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AN INVESTIGATION OF THE MACBETH ILLUMINOMETER[1]

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Abstract

The MacBeth illuminometer is a well known portable photometer of the visual type. Measurements of its errors show that the instrument gives reliable results if used with care. The most serious errors may occur when the color temperature of the light that is being measured differs greatly from that of the working standard lamp in the instrument. Under such conditions, different observers may obtain widely different results.

Introduction

The MacBeth illuminometer (3, p. 13; 7, p. 7) is a portable photometer of the visual type, well known to illuminating engineers and optical physicists. It is deemed unnecessary to describe the instrument in detail, because of its wide distribution, but attention should be drawn to the fact that a separate reference standard lamp permits frequent recalibration of the working standard in the instrument. Because of the conditions under which portable photometers are used, this provision adds greatly to the accuracy of the illuminometer.

Of late years, illuminating engineers have come to rely more and more on photoelectric photometers for portable use, and it is probable that these are amply accurate for their purposes if frequently checked. The MacBeth illuminometer, however, has found wide and increasing use in physical researches for many kinds of incidental photometry, and physicists have frequently used it in situations where an older generation would probably have had recourse to much more elaborate apparatus. In all of this they have displayed a confidence in the instrument possibly justified but not very well documented. These considerations led the authors to undertake a study of the MacBeth illuminometer.

A few statements are to be found in the literature regarding the accuracy of portable photometers in general. In their instructions for making illumination surveys (2) the I.E.S. (New York) assign a round figure of $\pm 5\%$ to such measurements. The British Standards Specification (1) for visual-type portable photometers demands an accuracy of $\pm 10\%$. A study by A. K. Taylor

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(6) is even more pessimistic, but in this he was concerned with a miscellaneous group of instruments, obviously not operated by experienced physicists. In another study (5) Taylor considered that a photometer of the general type that we are considering "should be capable of repeating readings to within 2 per cent" (5, p. 359). The departure of the test surface from the cosine law was studied in some detail by Taylor, who found this to be the most important source of systematic error.

Wetzel and Gouffé (8), on the basis of a careful analysis of all the sources of error, suggest a "mean probable error" (square root of the sum of the squares of the individual maximum errors) of 6% for an illuminometer of this type. In their analysis, the test plate contributes little to the total error.

Since the error due to test plates of various materials has been investigated thoroughly by Taylor (5), the present work was confined to three aspects of the problem:

(1) The error due to the necessity of adjusting the current through the working standard.

(2) Temperature errors in the milliammeter.

(3) Photometric errors.

In addition, some measurements of illumination were made indoors under practical conditions in order to make an estimate of the reproducibility of such measurements.

Errors Due to Current Adjustment

In the use of the MacBeth illuminometer, the current through the working standard lamp has to be adjusted to a certain value previously determined by photometry of a test plate illuminated by the reference standard. The adjustment is made by means of a rheostat and the current read on a milliammeter. The rheostat and meter supplied with the instrument are of high quality, the meter being provided with an antiparallax mirror.

To test the possible precision of this adjustment, a potentiometer was connected across a 0.1 ohm standard resistor placed in series with the rheostat, milliammeter, and lamp. The current could be read on the potentiometer to better than 0.1 ma. with ease. Settings were made on one of the divisions of the meter scale, and also on an arbitrarily chosen current (e.g. 228.5 ma.) between the divisions, which are at intervals of 5 ma. Care was taken to avoid parallax by placing the eye so that its image in the meter-scale mirror coincided with that of the pointer.

It soon became apparent that the rheostat itself is the limiting factor. At normal lamp currents, the passage from one turn of the rheostat to an adjacent turn changes the current by about 0.4 ma. or 0.2%. When the meter was being set on a line, the potentiometer readings indicated that one or other of two adjacent rheostat turns was almost always chosen. When it was being set between divisions, one of three turns would usually be taken. In either case, the uncertainty produced is of the order of 0.2%.

In the ordinary use of the illuminometer, the current is set to the nearest milliampere. In view of the properties of the rheostat, it is of doubtful value to attempt a closer setting than this, and we may therefore conclude that the maximum error that should be expected from this cause is 0.2% in current.

A variation of 0.2% in current represents a change of 1.5% in light output. This was determined for the working standard by photoelectric photometry, using a G.E. barrier-layer cell and 'zero-resistance' circuit (4). The cell with its correcting filter was tested for spectral response and found to approximate the visibility curve of the normal eye. Now there are three current settings involved in a complete series of measurements: (*a*) reference standard lamp current, (*b*) setting of working standard lamp current to make brightness match with reference standard, and (*c*) setting current through working standard during measurements. It is possible that the three errors might all be of the same sign, in which event their sum (expressed in light output) could reach 4.5%. The square root of the sum of the squares of the three errors is 2.6%, but note that the distribution of such errors is not at all normal.

Temperature Errors of Milliammeter

The milliammeter was calibrated at three temperatures by the Electrical Measurements Section of the National Research Council, through the kindness of Mr. A. J. Grant. It was found that a change of 10° C. in temperature produced an error of 0.2% in current, or 1.5% in light output. A decrease in temperature will cause the illuminometer to read high. Thus if the working lamp were compared with the reference standard indoors, and the instrument then taken outdoors for measurements, in winter, large errors might result.

Photometric Errors

The MacBeth illuminometer is comparatively seldom used to measure light at the same color temperature (about 2400° K.) as that from its working lamp. For this reason it is important to know the errors of heterochromatic measurements as well as homochromatic ones. It might be expected that measurements with a color match would be much more reproducible than those with a large color difference.

In this investigation, the color temperatures used were 2400°, 2840°, 3500°, and 6000° K., the two highest being obtained by the use of blue glass filters. The transmittance of these filters was carefully measured with a König–Martens spectrophotometer and the color of the light calculated in the usual way. The working lamp of the illuminometer was operated at a color temerature of 2440° K. throughout.

A magnesium oxide surface about 12 cm. square was set up normal to the axis of the photometer bench, and a well-aged lamp mounted on one of the movable carriages. The voltage on this lamp was controlled to ±0.02%, so that less than 0.1% scatter would be produced in the observations by voltage fluctuations. The magnesium oxide surface was observed at an angle

of about 45°, the illuminometer being clamped in position to minimize the fatigue of the observers in the rather long series of observations involved. In order to make sure that the clamping of the instrument did not alter the precision of the readings, 48 sets of 10 readings each were made, half with the illuminometer held in the hand. Each entry in Table I shows the percentage probable error* of the mean of 10 readings under the stated conditions. It will be seen that there is no systematic difference.

TABLE I

PROBABLE RANDOM ERROR (%) OF THE MEAN OF 10 SETTINGS OF A MACBETH ILLUMINOMETER, USING TWO METHODS OF SUPPORT

Color temp.	Illumination (about)					
	3 l. ft.$^{-2}$		10 l. ft.$^{-2}$		20 l. ft.$^{-2}$	
	Clamped	Hand-held	Clamped	Hand-held	Clamped	Hand-held
2440	0.40	0.57	0.44	0.85		
	0.74	0.47	0.66	0.83		
	0.61	0.47	0.62	0.57		
	0.55	0.28	0.54	0.44		
2840			0.99	1.00	1.02	0.92
			0.63	1.13	1.24	0.99
			1.04	0.89	1.06	1.00
			0.71	0.69	1.17	1.09
3500			1.28	0.97	0.80	1.00
			0.84	1.42	1.72	1.09
			1.33	1.13	1.38	1.15
			1.36	1.37	0.58	1.14

With the apparatus set up as described, two series of readings were taken, each on six successive days, a complete set covering both observers and four color temperatures being taken each day. In order that the error of reading the milliammeter should not enter, the current through the working standard was measured with a potentiometer as described above. At the beginning of each day's observations, the working lamp of the illuminometer was standardized by comparison with the reference standard.

The results of this investigation are summarized in Table II. It should be emphasized that each entry in this table is the result of 60 readings taken over six days, so that any variation in the actual value of the illumination will also be included. It is believed, however, that this was less than $\pm 0.1\%$.

It will be noted that in the homochromatic case, the difference between the observers was of the same order of magnitude as the probable error, but at the higher color temperatures the difference became much more important. No explanation is suggested for the much greater difference between the

* *The probable error of the mean of N readings is defined as* $P = 0.6745\sqrt{\frac{\Sigma v_i}{N(N-1)}}$.

TABLE II

PROBABLE ERROR (%) OF A SET OF 10 READINGS AT VARIOUS COLOR TEMPERATURES, DERIVED FROM SIX SERIES OF 10 READINGS EACH. WORKING STANDARD LAMP AT 2440° K.

Color temp. °K.	Observer A		Observer B		Difference 100 (A–B)/A, %
	Illumination l. ft.$^{-2}$	Probable error, %	Illumination l. ft.$^{-2}$	Probable error, %	
2440	2.86	±0.90	2.89	±1.34	−1.0
	10.08	0.42	10.10	0.93	−0.2
2840	11.41	0.49	11.28	0.32	+1.1
	18.3	0.64	18.3	1.88	0.0
3500	9.69	1.42	8.73	2.32	+9.9
	18.0	2.24	16.3	1.77	+9.4
6000	3.53	1.19	3.48	2.78	+1.4
	6.91	3.34	6.63	2.94	+4.0

observers at 3500° than at 6000° K., except that one or both of them must have adopted different criteria of equality at the two temperatures. There is no tendency towards greater precision at the higher of the two values of illumination.

The probable error of a single reading is about three times that of the set of 10 readings, assuming a normal error curve. Since a deviation 2.4 times as great as the probable error may occur once in 10 readings, it will be seen that at a color temperature of 3500° K., a single observation might be in error by about 15% once in 10 times. Even the average of a set of 10 readings might be in error by 5%. With a color match, this is reduced to 1% or at the most 2%.

Errors in Practical Use

The experiments described in the preceding section were made under laboratory conditions, corresponding to the type of use that is made of the instrument by many physicists. It was felt desirable to supplement these measurements by some observations of the kind that would be made by illuminating engineers in the course of a lighting survey, although it is realized that photoelectric photometers have largely replaced visual ones for such purposes.

A 500-watt frosted lamp was hung in a fixed position in a large room that could be darkened. This lamp was run from a storage battery and the voltage controlled to about 0.02%. Four stations were established in the room to permit the test plate to be placed in the same position on successive days. The walls of the room were cream-colored with a reflection factor of about 0.60, the color being approximately Munsell 5Y8/4, and most of the equipment in the room was black in color. The color of the illumination was therefore sensibly that of the light from the lamp, 2820° K.

Each day for six days the illuminometer was first checked against the reference standard, and then four readings were made by each of two observers at each of the four selected stations. The entries in the body of Table III are the means of these sets of four readings. The penultimate line in the table

TABLE III

DAILY MEASUREMENTS IN A ROOM ILLUMINATED BY A LAMP AT A COLOR TEMPERATURE OF 2820 K. AVERAGES OF FOUR READINGS ON EACH OCCASION. ILLUMINATION IN LUMENS PER SQUARE FOOT.

Day	Position							
	1		2		3		4	
	Observer							
	A	B	A	B	A	B	A	B
1	2.62	2.64	7.03	7.22	4.41	4.71	16.5	16.3
2	2.59	2.59	7.15	7.47	4.57	4.59	16.2	16.4
3	2.60	2.64	7.06	7.20	4.34	4.62	16.6	16.5
4	2.66	2.64	6.90	7.09	4.54	4.81	17.1	16.3
5	2.62	2.59	6.81	6.92	4.43	4.54	16.8	16.7
6	2.64	2.68	6.91	7.01	4.55	4.63	16.5	16.5
Mean	2.62	2.63	6.98	7.15	4.47	4.65	16.6	16.5
Probable error of four settings, %	0.66	0.89	1.20	1.82	1.41	1.40	1.25	0.66
Difference between observers, %, 100(A–B)/A	−0.4		−2.4		−4.0		+0.6	

gives the probable error of a set of four readings. It should be noted that this includes the error resulting from the daily standardization of the illuminometer lamp against the reference standard. The probable error is in all instances less than 2%, so that it becomes unlikely that the mean of four readings will be in error by more than 5%, as far as photometric errors are concerned.

Another series was run later as a check with the lamp at a color temperature of 2915° K., but the results are so similar that they will not be given.

Attention is directed to the considerable, and somewhat erratic, differences between the two observers. As the sets of observations were made at Positions 1 to 4 in immediate sequence, it is improbable that either observer would change his criterion of brightness match between the various positions. The good correspondence between the results for successive days makes such an explanation even more unlikely. It seems probable, therefore, that the greater part of the scatter in these differences is due to the obstruction of part of the illumination by the body of the observer. Each observer probably took, at each station, a stance that seemed comfortable, and repeated this fairly closely each day. It should be noted that this source of error also operates in the use of photoelectric illuminometers.

Conclusion

The conclusion that may be drawn from all these measurements is that the systematic errors are considerably more important than the random ones. Considered as a photometer apart from the external conditions of its use, the MacBeth illuminometer has excellent properties. Where only relative readings are required, and especially with light of a constant color, the mean of a few settings will almost certainly give a precision of $\pm 5\%$, and if an approximate color-match can be obtained, this can be improved to $\pm 2\%$. In laboratory use, the additional error due to the current setting can be reduced to negligible proportions by the use of a potentiometer and a standard resistance.

On the other hand, serious systematic errors can enter into the use of the instrument even under laboratory conditions, when light of high color temperature is being measured. It is desirable that the color vision of the observer should be known to be normal, or at least that his Y/B ratio (7, p. 263) should be nearly unity, before any reliance is placed on such measurements.

When it is necessary to measure illumination coming from several or all directions, an uncertainty is bound to arise from the obstruction of some of the light by the body of the observer. This can be minimized only with care in selecting a stance in relation to the incident light. The recommendations of Taylor (5) regarding the test plate should be followed. The test plate furnished with the illuminometer is of a material (pot opal glass) found by Taylor to be the most satisfactory of all materials studied. An angle of view of 30° to the normal was found to be the best. One may conclude that the figure for the precision, $\pm 5\%$, suggested by the I.E.S. (2) is of the right magnitude and certainly not too great.

Finally, the instrument should not be subjected to a large change of temperature between calibration and use.

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CANADIAN WILTSHIRE BACON

XXX. EFFECTS OF CURING AND COOKING ON THE THIAMINE, RIBOFLAVIN, AND NIACIN CONTENTS OF LONGISSIMUS DORSI MUSCLES[1]

By Dyson Rose[2] and Ruth Peterson[2]

Abstract

Laboratory studies on the thiamine, riboflavin, and niacin contents of longissimus dorsi muscles and of spent pickle indicate that there was little or no destruction of these vitamins during the curing process but that approximately 24% of the thiamine, 12% of the riboflavin, and 29% of the niacin were leached from the meat. The losses due to leaching increase with greater relative pickle volumes and are greater from an exposed surface of lean meat than from a surface covered with fat or rind. The commercial practice of rebuilding spent pickle probably reduces the over-all vitamin losses.

The losses of thiamine and of riboflavin incurred during the cooking of pork were found to be considerably greater than those incurred during the cooking of bacon. Niacin was lost to about an equal extent from both pork and bacon. The stabilization of the thiamine and riboflavin that occurred during the curing process was sufficient to offset the losses during cure and thus, after cooking, bacon was as good a source of these vitamins as pork.

Introduction

The B-vitamin content of pork has been estimated in several laboratories but the losses incurred during the conversion to bacon have not been studied extensively. Jackson and his coworkers (4) determined the retention of thiamine, riboflavin, and niacin following "wet cure" of bacon and found a loss of thiamine, niacin, and probably riboflavin, but the loss varied considerably from sample to sample. McIntire *et al.* (5) report values for the thiamine and niacin contents of "Canadian bacon" that are somewhat below the usual values for fresh pork, indicating that a loss may have occurred during processing. Schweigert and coworkers (11) have also reported losses of thiamine, riboflavin, and niacin from pork hams during cure. Working in these laboratories, Gorham (2) found lower thiamine, and niacin contents of Wiltshire half-backs after curing in pickle but found no significant change in the riboflavin content.

The results of numerous studies on the effect of cooking on the vitamin content of pork and pork products have indicated that 10 to 50% of the thiamine, 10 to 25% of the riboflavin and 0 to 20% of the niacin may be lost (1, 2, 9). Variations in the losses have been related to differences in the type of product studied and in the time, temperature, and method of cooking (4, 7, 8, 11). The studies of Rice *et al.* (8), of Nymon (6), and of Gorham (2) have indicated that the stability of these vitamins may be influenced by the

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admixture of other materials into the pork products and by the curing processes. The work of Gorham (2) appeared particularly interesting as it indicated that a change occurring during the curing of Wiltshire bacon stabilized the niacin against subsequent loss during cooking.

The studies reported in the present paper were undertaken to determine the fate of the vitamin lost from the meat, the effect of variations in the Wiltshire curing process on the extent of the loss, and the effect of commercial curing on the vitamin losses during subsequent cooking.

Analytical Methods

The methods used for estimating the vitamin content of the lean meat were essentially the same as those previously described (2), thiamine by the thiochrome procedure, riboflavin by a fluorometric method, and niacin by microbiological assay. At first, pickle samples were assayed without preliminary hydrolysis but it was found that hydrolysis increased the thiamine assay values, and an enzymic hydrolysis of a 1 : 10 dilution of the spent pickle was made for all subsequent thiamine assays. For convenience, niacin assays were also made on the hydrolyzed samples although the hydrolysis had no significant effect on the results. The 10-fold dilution, which was necessary to avoid salt inhibition of the hydrolytic enzymes, was too great to permit subsequent riboflavin assay. Hydrolysis with sulphuric acid was tried but the assays were of low accuracy owing to the high salt (sodium chloride plus sodium sulphate) concentration. All pickle samples were therefore assayed for riboflavin directly by a microbiological procedure, with sufficient dilution to avoid serious interference from the sodium chloride. The technique used was essentially that of Snell and Strong (12), but the basal medium was modified by doubling the yeast extract, tripling the dextrose, and adding 6 gm. of crystalline sodium acetate and 20 ml. of casein hydrolyzate (General Biochemicals Ltd.) per 500 ml. of basal medium.

Moisture and chloride determinations were made on the lean meat samples by methods previously described (3). The results of analysis of variance of the dry weight data did not differ essentially from those of the moist weight data, so the assay values, with the exception of those for the cooking experiment, are reported as μgm. per gm. of fresh or cured lean meat. For comparison the vitamin content of the pickle is also reported as μgm. per gm. of meat, the conversion from μgm. per ml. of pickle being made by multiplying by the pickle : meat ratio.

Experimental

Variability in Materials

Rice *et al.* (9) and others have shown that a considerable variation in vitamin content may occur from muscle to muscle in a single hog as well as in the same muscle from different hogs. In the present work the variability was reduced as much as possible by making all determinations on the longissimus dorsi muscles. Marked variations were found even within this muscle (Table I) although the variation between samples from one hog was less than

TABLE I

VARIATIONS IN VITAMIN CONTENTS (μGM. PER GM.) OF LONGISSIMUS DORSI MUSCLES IN FRESH PORK

Vitamin	Between 11 different hogs		Between 10 samples from the same hog	
	Range	Average	Range	Average
Thiamine	8.1 – 15.4	12.3 ± 2.1	13.2 – 13.8	13.5 ± 0.2
Riboflavin	1.30 – 1.76	1.50 ± 0.15	1.54 – 1.84	1.65 ± 0.11
Niacin	26.7 – 78.7	53.5 ± 4.5	35.3 – 42.2	40.9 ± 2.1

that between hogs. The data indicated a slight decrease in the vitamin content from the fore end to the back of the hog but the differences were significant (5% point) for riboflavin only.

Effect of Salt Concentration and Duration of Cure

To determine the effects of brine concentration and pickling time random sections of longissimus dorsi muscles weighing approximately 200 gm. each were immersed individually in brines containing 1% sodium nitrate, 0.05% sodium nitrite, and 15, 22.5, or 30% sodium chloride. Curing times of zero to seven days at 40° F. and a meat : pickle ratio of 4 : 3 (by volume) were used. All samples were ground shortly after removal from the pickles and were stored at −10° F. until analyzed. The spent pickle was stored at 30° F. until analyzed (about six weeks); prolonged storage did not significantly affect the assay values. The experiment was conducted in triplicate. Thiamine, riboflavin, and niacin were determined on the meat and spent pickle, and chloride and moisture determinations were made on the meat.

The chloride content of the meat increased with curing time and with pickle concentration, the final (seven day) values averaging 5.72, 7.25, and 9.02% for the meat from the 15, 22.5, and 30% brines respectively. Moisture content of the meat decreased with time and with brine concentration, being 73.1% in the fresh meat and 71.1, 69.4, and 67.5% after seven days cure in the 15, 22.5, and 30% brines.

Table II shows the average thiamine, riboflavin, and niacin contents of the meat and spent pickle together with the necessary differences for significance. The salt concentration did not significantly affect the distribution of thiamine or niacin but the amount of riboflavin found in the pickle increased with increasing salt concentration (average values 0.10, 0.13, 0.14 μgm. per gm. for the 15, 22.5, and 30% pickles respectively; necessary difference for significance, 5% level, 0.015).

These data show that although only the thiamine and niacin content of the meat decreased significantly during cure, all three vitamins accumulated in the pickle. Riboflavin, as well as thiamine and niacin, must therefore have been lost from the meat but the losses involved were small relative to the

TABLE II

DISTRIBUTION OF THIAMINE, RIBOFLAVIN, AND NIACIN (μGM. PER GM OF MEAT) IN PORK AND PICKLE DURING CURE

Days in pickle	Pickle	Meat	Total
Thiamine			
0	—	13.2	13.2
1	1.2	11.3	12.4
2	1.6	9.9	11.4
3	2.2	9.8	12.0
4	2.5	9.7	12.1
5	2.7	9.1	11.9
6	3.2	9.2	12.4
7	3.2	9.2.	12.4
Necessary difference[a]	0.46	1.49	—
Riboflavin			
0	—	1.46	1.46
1	0.06	1.36	1.42
2	0.09	1.39	1.48
3	0.12	1.36	1.47
4	0.14	1.46	1.60
5	0.13	1.23	1.36
6	0.16	1.31	1.47
7	0.17	1.40	1.57
Necessary difference[a]	0.023	—	—
Niacin			
0	—	57.3	57.3
1	7.9	41.6	49.5
2	9.4	36.0	45.4
3	11.4	36.1	47.5
4	12.5	38.0	50.5
5	15.7	41.9	57.7
6	16.6	38.0	54.6
7	15.7	34.3	50.0
Necessary difference[a]	3.6	11.0	—

[a] *Necessary difference for significance, 5% point.*

variability between samples and are thus masked when the assay values for the meat are considered. The average amount of vitamin in the pickle at the end of the seven day cure represented 24.2% of the thiamine, 11.8% of the riboflavin, and 29.0% of the niacin originally present in the meat. Since the total amount of each vitamin remained constant within the limits of experimental error, these figures also represent the approximate percentage loss from the meat under these conditions.

Effect of Rind and Fatty Tissue.

Three samples of spent pickle obtained from a commercial packer were assayed and were found to contain 1.1 μgm. of thiamine, 0.06 μgm. of riboflavin, and 3.1 μgm. of niacin per gram of meat cured. These results are lower than those obtained in the laboratory work and it appeared probable that the greater area of lean and cut surface exposed to the pickle during the laboratory cure had allowed a greater amount of vitamin to leach from the

meat. To test this assumption all but one of the lean surfaces of 10-in. central sections from the right sides of three hogs were covered with lard and the sections were immersed in 30% brine. At the end of a seven day curing period at 40° F. the sections were sliced longitudinally and the slices of longissimus dorsi, trimmed free of fat, were apportioned so as to give four samples of approximately 125 gm. each taken at increasing depths below the exposed surface. The left sides from the same hogs were similarly sliced when fresh to serve as controls.

The losses, expressed as average differences between the corresponding control and cured samples, are given in Table III. The loss of thiamine and niacin decreased markedly as the distance from the exposed surface increased, but the losses of riboflavin were too small to show significant variations.

TABLE III

AVERAGE LOSSES OF THIAMINE, RIBOFLAVIN, AND NIACIN FROM CURED PORK SAMPLED AT VARIOUS DEPTHS BELOW A SINGLE EXPOSED SURFACE

(Averages for three hogs, μgm. per gm. of meat)

Approx. depth, in.	Thiamine	Riboflavin	Niacin
0 – 0.4	5.6	0.07	34.0
0.4 – 0.8	2.2	−0.10	21.7
0.8 – 1.2	0.7	−0.10	10.1
1.2 – 1.6	1.0	0.03	7.5
Necessary difference, 5% point	1.31	—	9.88

Effect of Meat : Pickle Ratio

Six random sections of the longissimus dorsi muscles from each of three hogs were used to determine the effect of increasing the volume of brine relative to the meat. A brine of 1.0% sodium nitrate, 0.05% sodium nitrite, and 30% sodium chloride was used at meat : pickle ratios of 1.0 : 0.5, 1.0 : 0.8, 1.0 : 1.0, 1.0 : 1.3, and 1.0 : 2.0 by volume. The samples were cured individually for seven days and were then ground and stored at 40° F. until analyzed.

The thiamine, riboflavin, and niacin contents are given in Table IV. Significant quantities of thiamine and niacin, but not of riboflavin, were lost from the meat to the pickle. A slight over-all loss of thiamine occurred during this experiment. The losses of thiamine and of niacin increased considerably as the volume of pickle relative to the meat increased and the loss of riboflavin increased slightly but not significantly.

Effect of Rebuilding Pickles

In commercial practice, spent Wiltshire pickles are usually rebuilt and reused and an attempt was therefore made to assess the effect of this practice on the vitamin losses. The vitamin contents of a series of commercial pickles

TABLE IV

DISTRIBUTION OF THIAMINE, RIBOFLAVIN, AND NIACIN IN PORK AND PICKLE AFTER CURE AT VARIOUS MEAT : PICKLE RATIOS

(Averages for three hogs, μgm. per gm. of meat)

Meat : pickle ratio	Pickle	Meat	Total
Thiamine			
Fresh meat	—	11.2	11.2
1.0 : 0.5	2.7	8.1	10.8
1.0 : 0.8	2.8	7.3	10.1
1.0 : 1.0	3.2	7.4	10.6
1.0 : 1.3	3.9	5.9	9.8
1.0 : 2.0	4.1	6.0	10.1
Necessary difference[a]	1.03	1.36	0.78
Riboflavin			
Fresh meat	—	1.51	1.51
1.0 : 0.5	0.13	1.44	1.57
1.0 : 0.8	0.16	1.47	1.63
1.0 : 1.0	0.17	1.47	1.64
1.0 : 1.3	0.20	1.35	1.55
1.0 : 2.0	0.23	1.37	1.60
Necessary difference[a]	—	—	—
Niacin			
Fresh meat	—	55.0	55.0
1.0 : 0.5	10.6	35.0	45.6
1.0 : 0.8	14.8	35.2	50.1
1.0 : 1.0	17.6	36.3	53.9
1.0 : 1.3	19.5	34.3	53.8
1.0 : 2.0	19.0	30.1	49.1
Necessary difference[a]	3.25	9.22	—

[a] *Necessary difference for significance, 5% point.*

increased only slightly during reuse (for three consecutive batches, thiamine 1.1, 1.1, 1.2; riboflavin 0.05, 0.05, 0.07; niacin 2.8, 3.0, and 3.4 μgm. per gm. of meat). Some of the processes used to clarify and rebuild pickles were applied on a laboratory scale and the results are presented in Table V. These results do not necessarily correspond to those that would be obtained under commercial conditions but they indicate that much of the vitamin should be retained in the rebuilt pickle unless carbon black is used as a decolorizing agent.

Effect of Storage and Cooking

Six fresh (pork) and six cured (bacon) rib-in export backs were obtained from a commercial packing concern. These were cut into two pieces and the pieces were individually wrapped in wax paper, overwrapped with kraft, and stored at 30° F. Duplicate cured and uncured pieces were removed at weekly intervals and cut into slices 3 to 4 mm. in thickness. Alternate slices of the longissimus dorsi muscles, trimmed free of extraneous tissue and fat, were used to constitute the cooked and uncooked samples.

TABLE V

VITAMIN CONTENT OF PICKLE AFTER CLARIFICATION, μGM. PER ML.

Treatment of 500 ml. aliquots of pickle	Thiamine	Riboflavin	Niacin
Control	1.1	0.07	5.4
Slurried with 1.6 gm. 'Filter Cel' and filtered	1.1	0.04	5.2
Slurried with 1.0 gm. 'Norit' and filtered	0.4	0.02	1.3
Heated in boiling water bath 60 min., filtered	1.2[a]	Nil	5.7[a]

[a] *No correction applied for evaporation.*

The slices for the cooked sample were broiled until thoroughly done but not browned (500° F. for three minutes on one side, one minute on the other). Both samples were ground and stored at −40° F. until analyzed.

The moisture content of all samples tended to decrease during storage but the losses did not reach the level of statistical significance. During cooking the average moisture content dropped from 70.9 to 56.0% for the pork and from 71.7 to 61.7% for the bacon.

The riboflavin and niacin contents of the samples did not vary significantly during storage but the thiamine content increased irregularly during the first three weeks of storage and then fell again. Although this rise was statistically significant for both the fresh and dry weight data it was probably the result of random differences in the thiamine content of the backs as purchased.

The average vitamin contents of the meat samples before and after cooking are given, on a dry weight basis, in Table VI, together with the percentages

TABLE VI

THIAMINE, RIBOFLAVIN, AND NIACIN CONTENT OF UNCOOKED AND COOKED PORK AND BACON AND THE PERCENTAGE OF EACH VITAMIN RETAINED IN THE COOKED MEAT

(Averages for all storage times, dry weight basis)

	Bacon	Pork	Difference
Thiamine			
Uncooked, μgm. per gm.	56.7	51.3	+ 5.4
Cooked, μgm. per gm.	55.0	42.6	+12.4**
Retention, %	97.3	83.2	+14.1**
Riboflavin			
Uncooked, μgm. per gm.	5.85	6.60	− 0.75*
Cooked, μgm. per gm.	5.64	4.86	+ 0.78*
Retention, %	97.6	73.7	+23.9**
Niacin			
Uncooked, μgm. per gm.	193.1	269.7	−76.6*
Cooked, μgm. per gm.	174.9	228.7	−53.8*
Retention, %	91.3	88.3	+ 3.2

* *Significant to the 5% level.*
** *Significant to the 1% level.*

retained in the cooked samples. The retention of each of the three vitamins in the cooked samples was not significantly affected by storage, but, except for niacin, was greater in the bacon than in the pork.

Discussion and Conclusions

The data obtained during these experiments indicate that little or no destruction of the vitamins occurs during the curing of bacon. With the exception of thiamine in one experiment, all of the vitamin of the fresh meat was present in the meat and pickle at the end of the experiment. Vitamin losses from the meat are, therefore, due to leaching into the pickle. The extent of this leaching is influenced by the amount of exposed lean surface and the volume of pickle, but not, except with riboflavin, by the pickle concentration. The significant effect of pickle concentration on the riboflavin content of the spent pickle can not readily be explained.

Throughout the curing experiments no attempt was made to duplicate commercial practices and the figures for vitamin losses are not to be taken as representative of those incurred in packing plants. Gorham (2) has reported losses of 21.5% for thiamine, 2.7% for riboflavin, and 30.8% for niacin when half-backs were cured on a semicommercial scale using fresh pickle, and Jackson (4) reported average losses of 25.9% of the thiamine, 11.0% of the riboflavin, and 19.2% of the niacin during "wet cure" of bacon on a similar semicommercial basis. These figures do not differ markedly from those obtained in the present work using small pieces of lean meat (24.2% of the thiamine, 11.8% of the riboflavin, and 29% of the niacin). The additional data presented on the effect of the relative amount of pickle, and of the area of lean meat exposed to the pickle, indicate that these results are all high, and that commercial use of a maximum meat : pickle ratio, the curing of whole sides, and the use of rebuilt pickles probably reduce the losses materially.

The results of the cooking experiment are not in complete agreement with those of Gorham (2). The data show that the losses of thiamine and of riboflavin caused by cooking are significantly lower from bacon than from pork, thus confirming a trend suggested by the data of Gorham. But, on the other hand, no significant differences in the percentage of niacin retained in the cooked samples were found in the present work. The samples were cooked for a shorter time (four minutes) than that used by Gorham (six minutes) and the moisture content of the cooked samples was considerably higher (Table VII). With the exception of riboflavin in pork, the losses incurred during cooking were therefore not as great. The losses from bacon were particularly small and any effects of storage were not significant.

The data in Table VII show that the moisture content of the cooked samples was more variable than that of the uncooked samples but the variation is not excessive and the degree of cooking appears to have been satisfactorily controlled. It is interesting to note that there is a significant difference between the moisture content of pork and bacon after cooking for four

TABLE VII

MOISTURE CONTENT OF PORK AND BACON BEFORE AND AFTER COOKING

	Source of data			
	Gorham[a] (averages of duplicates)		Present work (single determinations)	
	No. of samples	Moisture, %	No. of samples	Moisture, %
Uncooked pork	4	73.5 ± 0.79	12	70.9 ± 2.45
Cooked pork	4	50.5 ± 1.50	12	56.0 ± 3.37
Uncooked bacon	10[b]	69.9 ± 1.71	12	71.7 ± 1.62
Cooked bacon	10[b]	48.7 ± 3.98	12	61.7 ± 3.02

[a] *Private communication.*

[b] *Data for 10 samples cured to contain a salt and nitrite content similar to that obtained commercially.*

minutes but not after cooking for six minutes. This difference between the data for the two cooking times makes it appear unlikely that the improved vitamin retentions in the cured samples that were cooked for four minutes are dependent upon the higher moisture retention. It seems probable that the Wiltshire curing process effects a certain degree of stabilization of thiamine, riboflavin, and niacin but the extent of the losses of these vitamins during cooking is influenced by the conditions used and pork and bacon do not necessarily respond equally to a change in the cooking procedure.

Rice *et al.* (7) have shown that the losses during the home cooking of cured ham were lower than those during the cooking of fresh hams. They related this difference to the less severe cooking conditions required with the cured meat. Our samples were cooked under rigidly controlled conditions and the differences found are entirely the result of changes effected in the meat by the curing process. It is conceivable that the differences observed by Rice and his coworkers may also have been due, in part, to a similar effect of the curing process.

The pork and bacon samples used by us were from different pigs and the data for the uncooked samples can not be used for estimating the losses of the vitamins during the curing process. They serve, however, to indicate the relative value of the pork and bacon as a source of these vitamins, and it is apparent from the data that the stabilization of thiamine and riboflavin effected by the curing process was sufficient to offset the losses incurred during processing of the bacon. The cooked bacon is thus a better source of these two vitamins than is the cooked pork. Niacin, however, was lost, during cooking under these conditions, to almost the same extent from both the pork and the bacon and the cooked bacon therefore appears to be a poorer source of this vitamin than the cooked pork.

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THE EFFECT OF BUILDERS IN HARD WATER DETERGENT SOLUTIONS[1]

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Abstract

The water-softening ability of soda ash, trisodium phosphate, sodium metasilicate, tetrasodium pyrophosphate, and sodium hexametaphosphate was investigated. Each builder possessed great water-softening power and in each case the reaction with hardness was immediate and the residual hardness from maximum softening was less than 1 grain per gallon of water. The effect in detergent solutions of builders at concentrations that had given maximum softening in the above tests was investigated by determining soil removal, degradation, and deposit of insoluble compounds after 10 launderings of standardly soiled flannelette. In these relatively high concentrations the alkaline builders gave inferior detergency to that of soap alone in hard water. The sequestering agent, sodium hexametaphosphate, gave results that were superior to soap alone in hard water and equal to that of soap alone in soft water. In every instance the addition of builder before soap produced the same effect as builder with soap.

Although considerable data have been reported on the use of builders in soft water detergent solutions, experimental work involving the use of hard water has been limited. Cobbs, Harris, and Eck (6, 8) have made a comprehensive study of various combinations of builders with soap using concentrations up to 0.37% (builder and soap) in water of 300 p.p.m. hardness. Carter and Stericker (4) confined their work to the use of silicates in water containing temporary hardness. Bolton (3) used a variety of builders and made a study of the soap requirement for sudsing. The present work was undertaken in the hope of contributing more information on the action of builders in hard water solutions. Preliminary investigation involved a study of the water softening powers of a wide variety of builders with consideration of the rate and the extent of water softening. Further work involved a study of the detergent efficiency of the builders plus soap using the concentrations of builders that had given maximum water softening action. Study was also made to determine whether it would be best to add the builder to the solution before the addition of soap, or whether the combined addition of builder and soap as in a 'built soap' would be equally satisfactory. Estimation of detergent efficiency was made from Launder-Ometer washings of standardly soiled fabric. The extent of soil removal was determined by reflectance readings and chemical degradation by cuprammonium fluidities. A rough estimate of the build-up of insoluble compounds was obtained from determination of the percentage ash after 10 washings.

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Experimental

The compounds used were sodium carbonate, trisodium phosphate, sodium metasilicate, tetrasodium pyrophosphate, and a sodium phosphate glass with a molar ratio of Na_2O to P_2O_5 somewhat greater than that corresponding to the empirical formula of $NaPO_3$. For convenience this latter compound will be termed 'sodium hexametaphosphate'. A synthetic hard water (3) that would be best representative of natural waters was used. Thus all experimental work was carried out in water containing hardness equivalent to 17.5 grains per U.S. gallon of calcium carbonate (300 p.p.m.) and a calcium: magnesium ratio of 2 : 1 in terms of calcium carbonate. A stock solution containing 35 grains per gallon calcium carbonate equivalent (600 p.p.m.) was prepared by dissolving 0.6035 gm. of 73.5% calcium chloride (c.p. grade) and 0.4060 gm. magnesium chloride ($MgCl_2 \cdot 6H_2O$, c.p. grade) in 1 liter of distilled water. The soap* used was believed to be representative of a neutral soap made from an average amount of saturated and unsaturated fatty acids. For hardness determinations a standard soap solution was prepared according to the procedure of the American Public Health Assocation (1) so that 1 ml. of the solution was equivalent to 1 mgm. of calcium carbonate.

The procedure for hardness determinations was such that all determinations were made as closely as possible to 60° C. The sample bottles (250 ml.) were supported in a wooden rack suspended in a water bath adjusted to 60° C. A burette was mounted above the rack to enable titrations to be made while the bottles were in the bath and thereby prevent much loss of heat during titrations. Preparatory to each determination of hardness, a 25.0 ml. volume of the stock solution of hard water (600 p.p.m.) was pipetted into a 250 ml. glass stoppered bottle. Freshly boiled and cooled distilled water was run in from a 25.0 ml. burette so that with the addition of the desired quantity of a stock solution of builder the total volume would be 50.0 ml. The bottle was placed in the rack for a 40-min. warming period before the builder solution was added from a 5.0 ml. graduated pipette. The bottle was shaken. After the desired interval of time, i.e., either immediately or after 30 min., standard soap solution was titrated into the bottle a small amount at a time. The bottle was shaken vigorously after each addition and soap solution was added until a lather lasted for five minutes when the bottle was placed on its side. To minimize any change of temperature, the bottle was placed horizontally on top of the rack during the time necessary for the observation of lather. The volume of the sample was increased by the addition of soap solution but since this change was due to 80% grain alcohol it was considered negligible. The volume of standard soap solution required to give a permanent lather when multiplied by 20 gives the total hardness as the equivalent of calcium carbonate as parts per million.

Ten wash tests were made in a Launder-Ometer (2) on samples (3 in. × 4½ in.) of standardly soiled flannelette.† Flannelette, a napped fabric, was

* *Colgate Palmolive Peet Golden XXX Powdered Soap.*

† *Prepared at the National Research Council of Canada, Ottawa. The soiling medium was a mixture of lampblack, Nujol, and lanoline applied from a trichlorethylene bath.*

chosen in order to offer a large surface for adsorption of insoluble matter that might be deposited from the detergent solutions. For each builder eight samples were prepared, four being subjected to treatment whereby the builder was added to the water before soap and four whereby the builder and soap were combined before being added to the water. For each set, half the samples were saved for measurement of soil removal and degradation and half for measurement of insoluble compounds. The latter samples were weighed before washing to enable the estimation of percentage ash based on the original fabric.

All washings were done in the presence of 0.50% soap at 60° C. in Launder-Ometer bottles containing 100 ml. (measured at 20° C.) solution. To standardize the procedure, as well as for convenience, stock solutions of water, builder, and soap were prepared. The method of mixing the solutions of builder and soap was (*A*) adding builder before soap and (*B*) adding builder at the same time as soap. In each case, the desired volume of water was measured by a graduated cylinder into a Launder-Ometer jar containing 10 rubber balls. The bottle was placed in the Launder-Ometer until the water reached 60° C. For Method *A*, 10.0 ml. of builder solution was pipetted into the bottle and the solution was mixed by slight agitation, then 10 ml. soap solution (at 60° C.) was added by pipette. For Method *B*, 10.0 ml. pipettes containing builder and soap were held so that the solutions from both pipettes ran into the bottle in a single stream. In either case the solution was mixed; the sample was added; the bottle was returned to the Launder-Ometer. At the end of the 15 min. washing period, the sample was put through a wringer, subjected to two three-minute rinses at 60° C. in water softened by builder, and one three-minute rinse at 60° C. in untreated water (300 p.p.m.), wrung, and allowed to dry.

It might be mentioned that the purpose of adding builder to the first two rinse waters was to prevent precipitation of soap in the washed fabric by hard water salts.

Controls of soap alone were as follows: 0.50% soap in distilled water; 0.50% soap in hard water; 0.73% soap in hard water (since in measurements of water softening ability it was found that 0.23% soap was required to precipitate the hardness).

Extent of soil removal was determined by use of the Hunter Multipurpose Reflectometer (9). Two readings were made from each sample and the results from duplicate samples were averaged. Chemical degradation was estimated from cuprammonium fluidities (8). An estimation of the amount of insoluble matter deposited from the detergent solutions was made by ashing the washed samples and by calculating the ash as percentage weight of the original untreated samples. Ashing was done in a muffle furnace at dull red heat to prevent fusion of phosphates and thereby permit combustion to be complete.

Data

Water Softening Ability

The volume of standard soap solution required to produce permanent suds is shown in Table I basing the concentrations of builders on the as-weighed basis as grams per milliliter of solution and in Fig. 1 basing the concentrations on the anhydrous basis. Titrations when soap was added immediately after the builder and when soap was added 30 min. after the builder were similar

TABLE I

STANDARD SOAP SOLUTION REQUIRED FOR PERMANENT SUDS IN 100 ML. HARD WATER (300 P.P.M. CALCIUM CARBONATE EQUIVALENT) CONTAINING BUILDER

Concentration of builder, gm./100 ml.	Standard soap solution for sudsing in presence of builder, ml.				
	Soda ash	Trisodium phosphate	Sodium metasilicate	Tetrasodium pyrophosphate	Sodium hexameta-phosphate
0.05	14.78				10.00
0.09	11.12				
0.10	11.09	3.86			6.45
0.15	11.32				
0.20	11.75	3.66		4.66	0.45
0.25					0.35
0.30	14.00	2.92			0.23
0.40		2.35		2.32	0.23
0.45	11.10				
0.50		1.59	12.80	0.98	
0.60	9.55	1.13		0.52	0.23
0.65		0.89			
0.67		0.80			
0.70				0.31	
0.73		0.73			
0.75	8.89	0.73	9.00		
0.80		0.73		0.16	0.23
0.85				0.10	
0.90	7.81			0.05	
1.00		0.73	6.25	0.05	
1.50	5.55		2.46		
1.75			1.04		
2.00	3.93		0.66		
2.25			0.56		
2.50	1.64		0.47		
2.60	1.60				
2.70	0.80				
2.75	0.60		0.47		
3.00	0.60		0.47		
3.50	0.60				
4.50			0.47		

in every instance, consequently only the single values are listed. It is to be noted that for each builder there was a decrease in the amount of soap required for sudsing with increase of builder concentration until a concentration was reached beyond which the amount of soap required for sudsing did not change. The completeness of the water softening varied with the builders. Data pertaining to the lowest concentrations of the builders required to give maximal softening are given in Table II. It is to be noted that the residual hardness

ranged from 1 p.p.m. using tetrasodium pyrophosphate to 15 p.p.m. using trisodium phosphate. The concentration of builder required for maximum softening increased in the following order: sodium hexametaphosphate, trisodium phosphate, tetrasodium pyrophosphate, sodium metasilicate, and soda ash. On the anhydrous basis the trisodium phosphate concentration is

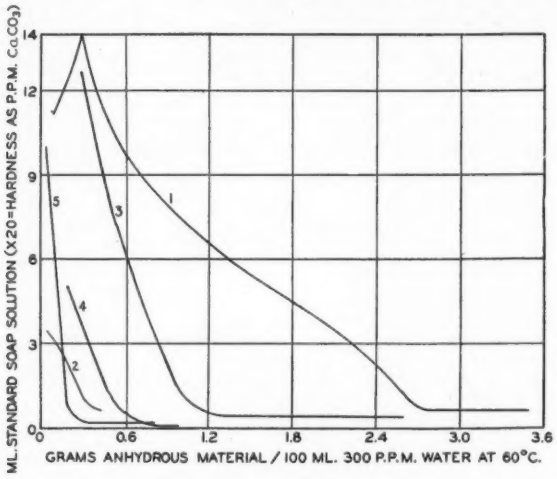

FIG. 1. *Standard soap solution required for permanent suds in 100 ml. water at 60° C. (300 p.p.m. calcium carbonate equivalent hardness) containing builder.*
1. Soda ash. 2. Trisodium phosphate. 3. Sodium metasilicate. 4. Tetrasodium pyrophosphate. 5. Sodium hexametaphosphate.

TABLE II

LOWEST CONCENTRATION OF BUILDERS ADDED TO HARD WATER (300 P.P.M. CALCIUM CARBONATE EQUIVALENT) FOR MAXIMUM SOFTENING

Builder	Residual hardness, p.p.m.	Concentration of builder, gm./100 ml.		No. of equivalent weights of builder: 1 equivalent weight of hardness*
		As weighed	Anhydrous	
Soda ash	12	2.75	2.75	86
Trisodium phosphate	15	0.73	0.32	10
Sodium metasilicate	9	2.50	1.44	40
Tetrasodium pyrophosphate	1	0.90	0.90	22
Sodium hexameta-phosphate	5	0.30	0.30	5**

** Obtained by dividing the number of equivalent weights of builder per liter solution by the number of equivalent weights of hardness per liter solution (0.006).*

*** In the absence of data regarding the exact composition of Calgon the formula was assumed to conform to that of sodium hexametaphosphate.*

practically equal to the sodium hexametaphosphate concentration and the concentration of sodium metasilicate appears appreciably lower than that of soda ash. It is interesting to note the wide variation in the ratio of the number of equivalent weights of builder to one equivalent weight hardness. These ratios are in agreement with the arrangement of builders according to builder concentrations.

A factor of considerable importance is the manner of softening the water. Soda ash, trisodium phosphate, and sodium metasilicate caused the removal of hardness by precipitation. Tetrasodium pyrophosphate removed hardness by formation of precipitates and soluble complexes depending on the relative amounts of phosphate and hardness. Sodium hexametaphosphate formed soluble complexes with the hardness.

Soil Removal

The extent of soil removal based on the increase of brightness over the reflection readings of the original soiled cloth are given in Table III. An

TABLE III

DETERSIVE EFFICIENCY DATA BASED ON INCREASE OF BRIGHTNESS AFTER 10 WASHES

Substance	Units of brightness increased		Efficiency (0.50% soap in distilled water = 100)		Efficiency index (efficiency: concentration)	
	Builder added before soap	Builder added with soap	Builder added before soap	Builder added with soap	Builder added before soap	Builder added with soap
I. *Builder solutions*						
Soda ash	10.7 ± 1.5*	11.3 ± 0.6	46	48	0.5	0.5
Trisodium phosphate	16.5 ± 0.3	16.9 ± 1.1	70	71	7	7
Sodium metasilicate	13.1 ± 0.3	13.2 ± 0.5	56	58	1	1
Tetrasodium pyrophosphate	15.9 ± 0.4	16.7 ± 0.5	68	71	3	3
Sodium hexametaphosphate	21.6 ± 0.2	21.6 ± 0.5	92	92	18	18
II. *Controls*						
0.50% soap in distilled water	23.5 ± 1.3		100			
0.50% soap in hard water (300 p.p.m.)	18.2 ± 0.5		78			
0.73% soap in hard water (300 p.p.m.)	18.7 ± 0.8		80			

* *Standard error.*

analysis of variance made on the results shows that the difference between Methods *A* and *B* is not significant; the difference between treatments is significant. Making allowance for standard error it is apparent from Table III that the difference between the distilled water control and the sodium hexametaphosphate treatment is not significant whereas the difference between

the distilled water control and any of the other treatments is significant. The treatments tend to take the following order of decreasing efficiency: 0.50% soap in distilled water, sodium hexametaphosphate, 0.50% soap in hard water, 0.73% soap in hard water, trisodium phosphate, tetrasodium pyrophosphate, sodium metasilicate, and soda ash. By placing the increase in brightness of the distilled water control at 100 and calculating the other values accordingly, the efficiencies of the solutions may be rated. An economical builder from the standpoint of detersive efficiency is one that gives a high efficiency when used in low concentration. A comparison of the builders based on both efficiency and concentration is given by the efficiency index obtained by dividing the efficiency by the number of equivalent weights of builder that reacted with one equivalent weight of hardness in water softening (from Table II). The efficiency indices show differences among builders having similar values for increase in brightness; trisodium phosphate has an efficiency index higher than that of tetrasodium pyrophosphate; sodium metasilicate has an efficiency index higher than that of soda ash. The superior position of sodium hexametaphosphate above the other builders is emphasized by the efficiency index.

Degradation

There was no significant difference between the fluidity of the original untreated material and that of any of the washed samples. Therefore chemical degradation and tensile strength loss from each washing procedure for 10 washings must be considered nil.

Deposit of Insoluble Compounds

When allowance is made for experimental variation, it becomes apparent from Table IV that the manner of addition of builder and soap did not affect the percentage ash. On the basis of percentage ash, the treated samples may be divided roughly into three groups: 0.50% soap in distilled water, and sodium hexametaphosphate; 0.73% soap in hard water, 0.50% soap in hard water, and tetrasodium pyrophosphate; trisodium phosphate, sodium metasilicate, and soda ash. Factors that might influence the ash of the samples are the fabric, the soil, and deposits from the detergent solutions. Since the washing procedure causes removal of soil and consequently a decrease in amount of ash attributed to the soil, any increase in ash must be caused by the detergent solution. The actual percentages of ash found by experimentation are in agreement with the manner of water softening by the substances concerned. For example, sodium hexametaphosphate, which formed a low percentage ash, formed soluble complexes with the hardness of water, whereas trisodium phosphate, sodium metasilicate, and sodium carbonate, which formed high percentage ashes, formed precipitates with hardness. Comparison with the controls of soap in hard water shows that the deposits formed from solutions of trisodium phosphate, sodium metasilicate, and sodium carbonate (i.e., calcium and magnesium hydroxides, phosphates, silicates, and carbonates) were considerably greater than the deposits from soap alone (i.e.,

lime and magnesium soap). This difference is typical of the behavior of inorganic and organic compounds. The higher ash from these builder solutions might also be caused by precipitates formed by builders with hardness in the first two rinses. The ash caused by tetrasodium pyrophosphate was

TABLE IV

ASH OF SAMPLES AFTER 10 WASHINGS

Substance	Ash, % of original weight	
	Builder added before soap	Builder added with soap
I. *Builder solutions*		
Soda ash	1.94	2.13
Trisodium phosphate	1.46	1.93
Sodium metasilicate	1.98	1.94
Tetrasodium pyrophosphate	0.89	0.64
Sodium hexametaphosphate	0.35	0.49
II. *Controls.*		
0.50% soap in distilled water	0.11	
0.50% soap in hard water (300 p.p.m.)	0.65	
0.73% soap in hard water (300 p.p.m.)	0.57	
Original material	0.20	

slightly higher than that by sodium hexametaphosphate yet considerably lower than that by the other builders. The explanation for this may lie in the action of tetrasodium pyrophosphate in the washing solutions. Solutions of tetrasodium pyrophosphate and hard water remained clear, probably owing to softening of water by dispersion or by sequestration of hardness. Addition of 0.50% soap to such solutions caused a cloudiness due to formation of a precipitate. Therefore it is possible that a precipitate was deposited on the fabric during washing and was not removed in rinsing operations.

The data show that the builders possessed great water softening ability and that the action in each instance was so rapid that the effect of the addition of builder before the soap was the same as that of the combined addition of builder and soap. With the exception of sodium hexametaphosphate, the builders gave results that were inferior to that from use of soap alone in Launder-Ometer tests.

Discussion of Data

In the determinations of water hardness no attempt was made to adjust the pH of the solutions. With the exception of sodium hexametaphosphate, which is nearly neutral, the substances would give pH values definitely on the alkaline side. It is possible that in alkaline solution, sodium hexametaphosphate might have lowered the final water hardness. The fact that less sodium

hexametaphosphate than tetrasodium pyrophosphate is required to remove hardness has been reported by Gilmore (7).

In the washing tests, with the exception of sodium hexametaphosphate, the builders in concentrations that were capable of giving maximum water softening did not improve the efficiency of soap alone in hard water. It is possible that these same agents did not possess great softening power under the conditions that existed in the washing tests, i.e., in the presence of 0.50% soap, in fact the agents might have given up the precipitated hardness to the soaps thereby precipitating them and reducing the washing efficiency of soap. Evidence that the maximum softening dosage of tetrasodium pyrophosphate did not hold in the presence of much soap is that this agent kept the water clear in the absence of soap but in the presence of considerable soap a precipitate formed. The same action might occur in the other alkaline baths but it would be masked by the alkaline precipitates. If this action were carried to completion the conditions for precipitation of hardness would be comparable to those existing in the solution of soap alone in hard water, i.e., the hardness would be precipitated solely by soap, consequently any deposit of insoluble material would be attributable to lime and magnesium soaps. However, in the presence of alkaline builders the percentage ash was greater than that produced with soap alone. Thus in the solutions containing alkaline builders the precipitation of hardness must be due at least in part to reaction with builder.

It is interesting to note that the actions of 0.50% soap and 0.73% soap in hard water were similar. Since 0.23% soap was probably used to precipitate the hardness it appears that, for detergent action after removal of hardness, 0.27% soap was as efficient as 0.50% soap. This is in agreement with work in soft water, which has indicated that soap concentrations beyond 0.25% are no more effective than that concentration (10).

In connection with measurement of chemical degradation it should be pointed out that the number of washings was of necessity limited to 10 and consequently no definite conclusions on the effects of these substances over long periods of washing can be drawn.

In relating the results of this work to large-scale operations, it seems probable that the same tendencies would be observed although the quantitative effects might not be the same; for instance, the build-up of compounds might not be as great since mechanical action would be more severe. It should be borne in mind that the concentrations of builders were those that gave maximum water softening and might not be the optimum concentrations for detergent efficiency. In fact, in the concentrations that were used the precipitation by builders reduced the detergent efficiency even more than the precipitation of lime and magnesium soaps alone. Harmful precipitates can be avoided by the use of a sodium phosphate glass, commonly termed sodium hexametaphosphate. The practice of using a 'built soap' would seem to be comparable to the technique of adding builder to the solution prior to the addition of soap.

Acknowledgments

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AN APPARATUS FOR DETERMINING THE SENSITIVITY OF GAS DETECTOR PAPERS[1]

A. S. TULK,[2] L. H. CRAGG,[3] AND R. P. GRAHAM[3]

Abstract

There is described an apparatus for determining the sensitivity of detector papers toward gases from liquids of low volatility. The paper holder is constructed in such a way that the papers may be moistened, dried, removed, or replaced without disturbing the flow of gas through the rest of the apparatus. The holder also makes possible the comparative testing, under identical conditions, of a number of papers. With auxiliary apparatus, the paper holder permits the use of a carrier air stream of controllable relative humidity.

Introduction

This apparatus was constructed and used to determine the sensitivity, to toxic agents of low volatility, of detector papers prepared by the impregnation of filter paper. Minor alterations in the auxiliary apparatus should permit its use with highly volatile agents. In operation, a stream of dry nitrogen or other inert gas is bubbled through the liquid toxic agent in such a way as to saturate the gas at the desired temperature. The saturated gas is diluted in a mixing chamber with air of controlled humidity. This diluted stream then passes in succession through a number of separate sheets of the detector paper under test.

Apparatus

The apparatus is illustrated in Fig. 1. The paper holder proper, *B*, is constructed of 22 mm. tubing (O.D.), its jacket of 40 mm. tubing bearing a 45/50 ground glass joint. The upper part of the jacket is ring-sealed to the main tube below the stopcock *A*. The lower part of the jacket is drawn off about 15 cm. below the joint and sealed to one arm of a U-tube, the other arm of which connects to the line by means of a 14/35 ground glass joint, *C*. (This U-tube is the only part of the apparatus that is constructed of 10 mm. tubing; the rest of the connecting tubing is 8 mm.) The 22 mm. tubing of the holder projects downward from the ring seal, passing about 2 cm. beyond the male member of the 45/50 joint. The end of this tube is ground square, polished, and fitted with hooks as illustrated. A 12 cm. length of 25 mm. tubing, selected to fit very snugly over the 22 mm. tubing has inserted into it a number of 15 mm. spacers of the 22 mm. tubing, the lowest of which is cemented into place.* (This is shown as a solid, heavy line in the drawing).

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* *General Electric Glyptal Cement was found satisfactory for this purpose.*

These spacers are cut square on the ends and polished to assure smooth fitting. The polishing was carried out on a wet felt disk charged with chromic oxide.

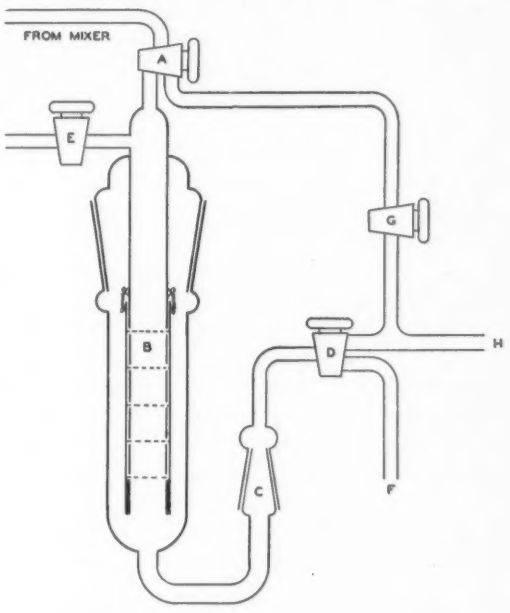

FIG. 1

In assembling the holder, a disk of detector paper (represented by a dotted line in the drawing) is inserted between each pair of spacers. The 25 mm. tubing, thus packed, is then slipped over the projecting end of the 22 mm. tube, the joint greased, and the springs fixed in place. The lower part of the jacket with its connecting U-tube and 14/35 joint is then clamped in place.

Operation

A stream of air of controlled humidity and controlled concentration of toxic agent enters the paper holder through the three-way stopcock, *A*. The moisture content of this air stream is adjusted by passing the air through a humidifier in parallel with a drier. The humidifier is composed of a glass jacket filled with 8-mesh Haydite* kept saturated with water. The drier is a second tube filled with 14-mesh silica gel.** By varying the relative rate of air flow through these two tubes, it is possible to obtain relative humidities (measured by means of a wet and dry bulb hygrometer) ranging from 30% to 95%. This air, of known humidity, is then passed through a

* *An expanded shale aggregate, processed by the Cooksville Company, Cooksville, Ont.*
** *Davidson 'Protex-Sorb'.*

flow meter and is led into a 1000 ml. bulb that acts as a mixer. Should completely dry air be required, the humidifier, drier, and hygrometer are by-passed by a phosphorus pentoxide drying tube constructed of a number of small tubes arranged in a larger tube and held in place by wax, the completed unit resembling a tubular boiler. The insides of the smaller tubes are covered with the drying agent and the inlet to the device is baffled to ensure passage of air through all the smaller tubes.

Into the mixer there also passes a stream of nitrogen saturated with the vapor of the reagent being used. This nitrogen (the commercial product) is dried thoroughly by being passed successively through calcium chloride and phosphorus pentoxide. The gas then passes through a flow meter and into a thermostated saturator similar to one described elsewhere (1).

The effluent from the mixer, which now contains a known concentration of water vapor and toxic agent, is passed into the paper holder as shown in the drawing. The air is drawn through the apparatus at a controllable rate by suction.*

As may be seen from the drawing, it is possible to load the paper holder with papers to be tested and then, by suitable manipulation of the stopcocks before admitting dry nitrogen to the saturator:

(*a*) to dry or moisten the papers as desired either before or after a run by passing dry or water saturated air through the path *E-B-C-D-F*,

(*b*) to bring the relative humidity of the diluent stream to the desired value through the by-pass *A-G-H*, without disturbing the papers in *B*,

(*c*) to adjust the flow rate to the desired value, and to balance the resistance in the by-pass by means of the stopcock *G* to that of the papers in *B*. This allows a duplicate run to be made, after reloading the paper holder *B*, without disturbing the flow rates.

When these adjustments have been made, the flow of gas may be regulated through the by-pass *A-G-H*, without being brought into contact with the papers. The flow is then directed through the paper holder by the path *A-B-C-D-H*, and the timing of the run begun. At the conclusion of the run, the metered flow is again diverted through the by-pass while the paper holder and the exposed papers are swept out with air through the path *E-B-C-D-F*.

It may be noted that a paper holder of this construction may be used to obtain information on the gas pickup per paper by passing the gas through several papers in series and noting the time of stain appearance on successive papers. Further, the use of papers in series has proved desirable for purposes of comparison.

Experimental

The results of an investigation of the sensitivity of two different detector papers to a toxic agent of low volatility are shown in Table I. Exposure of these papers to the vapor of the agent caused them to change color, and the

* *An Electrolux cylinder (Model ZB 30) with a rheostat to control its motor speed was found to be suitable for this purpose.*

purpose of the experiment was to determine whether or not the relative humidity of the air stream carrying the agent had any effect upon the sensitivity of the paper. For the data of Table I the following conditions obtain:

Flow rate of saturated stream (nitrogen) . . . 2.01 ± 0.05 ml. per min.
of diluent stream (air) . . . . . 1070 ± 70 ml. per min.

Relative humidity of the nitrogen stream (dried over phosphorus pentoxide) . . . . . zero

The papers were 'conditioned' before exposure by passing water saturated air through them for 15 min.

TABLE I

R. H.* of air stream, %	Time of color appearance	
	Min.	Average, min.
Paper No. 1		
0	18, 15, >26, >30	>15
33 ± 1	9, 6, 5	6.6
50 ± 1	4.6, 4.8	4.7
64 ± 1	7, 6	6.5
95 ± 1	>26, >26	>26
Paper No. 2		
0	>26, >26	>26
33 ± 2	10, 11, 10, 10	10.2
50 ± 1	3.7, 3.9	3.8
63 ± 1	5, 3.4, 3, 3.8	3.8
95 ± 1	5, 4.7, 5	4.9

* *R.H. = relative humidity.*

If the saturator is assumed or known to be 100% efficient and the vapor pressure of the toxic agent at the temperature of the saturator is known, it is possible to calculate from the data the sensitivity of the detector paper expressed in terms of the weight of agent required to change the color of unit area of paper.

Discussion

It will be observed that the sensitivity of a detector paper to a toxic agent may be a function of the relative humidity of the air stream in which the agent is dispersed. Paper No. 1 has a considerably lower sensitivity at high and low humidities than it has at intermediate humidities. The maximum sensitivity of the paper would appear to be obtained when the relative humidity is about 50%. Paper No. 2, however, is almost as sensitive at 95% relative humidity as it is at 50%. Like Paper No. 1, its sensitivity at low relative humidity is very poor.

These phenomena make the evaluation of detector papers at different humidities very necessary if the true efficiency of the paper is to be determined.

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INFLUENCE OF ORGANIC MATTER ON THE GERMICIDAL EFFICIENCY OF QUATERNARY AMMONIUM AND HYPOCHLORITE COMPOUNDS[1]

C. K. JOHNS[2]

Abstract

Using *Staphylococcus aureus* and *Eschericha coli* as test organisms, the influence of various concentrations of skim milk on the germicidal potency of Roccal and of Dalglish hypochlorite solutions was studied. Both germicides retained their activity in the presence of unexpectedly high concentrations of skim milk, especially against *S. aureus*. Small concentrations frequently showed a slight potentiating effect in both laboratory and plant tests. The effectiveness of the hypochlorite fell off sharply beyond a certain concentration, while that of Roccal declined more gradually. Solutions of Roccal prepared with tap water were decidedly less active against *E. coli* than those prepared with distilled water. With the hypochlorite, tap water solutions were equally effective. Against *S. aureus*, a similar difference was noted although to a lesser extent. Added skim milk depressed the germicidal action of tap water solutions of Roccal to a greater extent than for distilled water solutions, while for the hypochlorite the reverse held true.

Introduction

In the sanitizing of equipment and utensils used in the handling and processing of food, considerable emphasis is usually laid upon the necessity of first removing all organic residue from the surfaces to be treated. While certain disinfectants, such as phenol, are effective in the presence of high concentrations of organic matter, the activity of others, such as the hypochlorites, is sharply reduced. Little published work has appeared, however, concerning the effect of low concentrations of organic matter, such as might be encountered by a solution used to sanitize food equipment. Prucha (13) showed the decline in available chlorine content when skim milk was added to several types of hypochlorite solution. Mallmann and Edwards (11) reported that a 20 p.p.m. solution of chlorine water, to which 0.2% skim milk had been added, was almost devoid of germicidal activity against *E. coli* after holding for 19 hr., even though it showed 10.4 p.p.m. chlorine by titration. They therefore warned against the re-employment of used chlorine solutions in the dairy industry, and in consequence their use was forbidden under the Standard Milk Ordinance of the U.S. Public Health Service (14).

In our studies with chlorine sterilizing agents in 1933 and 1934 (7, 8), a glass slide technique was developed in which the test organisms were present in a film of diluted skim milk on the surface of a sterile glass slide. When partially dried, the slide was gently agitated in the germicidal solution for the desired period, then plated. Despite the presence of the film of skim milk, almost complete destruction of the test organism was effected by the

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more active hypochlorite solutions within a few seconds. Similar results have been obtained in more recent studies comparing hypochlorites with quaternary ammonium compounds (9). Under the conditions of these tests, therefore, the presence of the film of skim milk did not depress germicidal activity significantly. This has raised some doubt as to the extent to which small amounts of organic matter, such as would be encountered on carelessly cleaned equipment, might interfere with the germicidal activity of both hypochlorite and quaternary ammonium solutions. The present studies were undertaken to throw more light on this point.

Methods

An alkyl dimethylbenzylammonium chloride (Roccal) was selected as a representative quaternary ammonium compound, while Dalglish Liquid Bleach (containing 10 to 12% available chlorine) represented the hypochlorites. During the first part of these studies, working solutions were prepared by diluting the original products with sterile distilled water. When it was subsequently found* that tap water solutions of quaternary ammonium compounds gave results quite different from those obtained from distilled water solution, Ottawa tap water was substituted as being more representative of the diluent used in plant practice. All concentrations were expressed as parts per million of active ingredient or available chlorine respectively.

Based on our previous findings (9), *S. aureus* (F.D.A. No. 209) and *E. coli* No. 117 were selected as representative test organisms. All testing was carried out with solutions at 20° C. Except where indicated, the glass slide technique previously described (9) was used, with the following slight modifications: (*a*) the slide, after agitation in the test solution for the required period, was rinsed in tap water for five seconds to minimize the carry-over of bacteriostatic concentrations of Roccal, and (*b*) the count on control slides was obtained by shaking the slide in 99 ml. of sterile saline, plating the slide itself and aliquots of the rinse, and calculating the total number originally present. (Studies over a considerable period showed that the five second rinse of the control slide in tap water removed an average of 81% of the number of cells of *S. aureus* originally present.) Bacto tryptone glucose agar (1) containing 7.5% sodium chloride to inhibit contaminants (3, 10), was used with *S. aureus*, and Bacto nutrient agar with *E. coli*. Plates were counted after incubation at 32° C. for 48 hr.

Skim milk was selected as the organic matter to be added, largely because of its homogeneity, uniformity from day to day, and of its representativeness of the type of residue likely to be encountered in the dairy industry. Comparative tests with homogenized and ordinary whole milks have given essentially the same results. Different results can be expected where other types of organic matter are used; preliminary tests with beef peptone broth, peptonized

* *The author is indebted to Mr. C. T. Butterfield, U.S. Public Health Service, Cincinnati, Ohio, for the information that different water supplies gave different results with quaternary ammonium compounds.*

milk, and sodium caseinate solution have shown all three to exert a greater depressing influence on the germicidal efficiency of Roccal than does skim milk, while peptone and gelatin are more nearly comparable to skim milk.

During the earlier studies, concentrations of Roccal and Dalglish were selected that would give approximately equivalent killing in the control solutions containing no added skim milk. In this way, it was felt that differences in reaction to added skim milk could be more readily observed. However, it subsequently became evident that the results of tests using 25 p.p.m. might differ significantly from those obtained at 100 or 200 p.p.m. Consequently, a further series of comparisons was made with both germicidal solutions at the same initial strength. These solutions were prepared from tap water instead of distilled water as in the previous tests, in order to simulate plant practice.

Experimental

Preliminary tests comparing Roccal 200 p.p.m. against Dalglish 50 p.p.m. led to the surprising discovery that the germicidal activity of the Roccal solution against *E. coli* was actually stimulated slightly by the addition of small amounts of skim milk. This aspect was therefore studied more exhaustively.

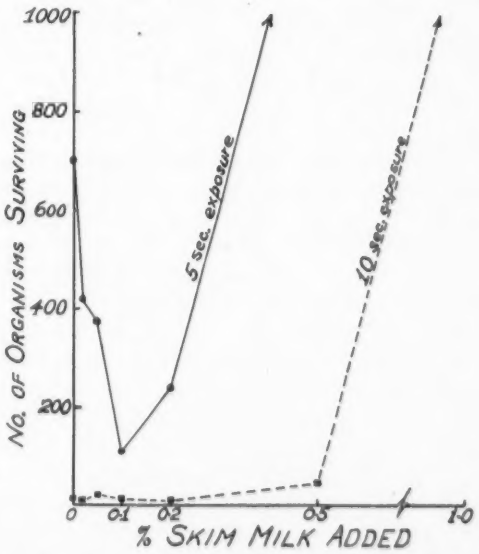

FIG. 1. *Survival of* E. coli *as influenced by addition of skim milk to Roccal solution (200 p.p.m.). (Each point represents average of triplicate determinations.)*

The results from a typical experiment appear in Fig. 1, wherein each point represents the average count from triplicate slides treated consecutively.

To check these findings, additional tests were conducted using a plating technique. In this, 1 ml. of standardized bacterial suspension was introduced

into 100 ml. of germicidal solution, kept agitated by a mechanical stirrer. At short intervals, 1 ml. portions were withdrawn, delivered into Petri dishes and immediately poured with agar medium. Several series of such tests were carried out, but as shown by the data from one of these (Table I) only a very slight potentiating effect could be detected. At concentrations up to 0.05%,

TABLE I

EFFECT OF ADDED SKIM MILK ON GERMICIDAL EFFICIENCY OF ROCCAL (50 P.P.M.) AS JUDGED BY THE PLATING TECHNIQUE. INOCULUM, *E. coli*. 7,500,000 CELLS PER ML. OF GERMICIDAL SOLUTION

% added skim milk	Count of survivors after exposure period, sec.					
	10	20	40	60	90	120
0	**18**	0	0	0	0	0
0.02	**3**	0	0	0	0	0
0.05	**258**	95	8	0	0	0
0.1	++++	+++	**1000**	76	6	0
0.2	++++	++++	++++	++++	++++	++++
0.5	++++	++++	++++	++++	++++	++++

N.B. *Plus signs indicate relative density of colonies.*
Bold-face type indicates point at which 99.9% destruction was noted.

the retarding influence was slight. At 0.1% (the concentration which showed maximum stimulatory effect by the glass slide method) a marked reduction in potency was indicated by the plating method. The lower concentration of Roccal employed in the latter (50 p.p.m. vs. 200 p.p.m. in the glass slide method) doubtless explains this difference; tests with 25 p.p.m. Roccal showed a definite retardation with only 0.02% added skim milk.

Attention was next directed to the behavior of these solutions against *S. aureus*. The initial studies showed that the addition of skim milk to Roccal had much less effect with this organism than with *E. coli*. With the glass slide technique no potentiating effect was observed with concentrations of skim milk between 0.02 and 1.0%. Since even 1% had little effect upon germicidal activity, higher concentrations were included in subsequent tests, in which both glass slide and plating methods were employed (Table II). Although the number of organisms subjected to germicidal action was proportionately much greater in the plating technique, and the concentration of Roccal was only one-fifth of that in the glass slide tests, the percentage survival, except in the presence of the higher concentrations of skim milk, was significantly lower. Nevertheless, both methods agree in indicating that the presence of skim milk in fairly high concentrations has little effect upon the ability of Roccal to destroy *S. aureus*. Having established this point, a further series of comparisons between Roccal and the hypochlorite was

TABLE II

EFFECT OF ADDED SKIM MILK ON GERMICIDAL EFFICIENCY OF ROCCAL IN DISTILLED WATER AS JUDGED BY PLATING AND GLASS SLIDE TECHNIQUES. *S. aureus*

% added skim milk	Plating (20 p.p.m.)[a] Count of survivors after exposure period, sec.			Glass slide (100 p.p.m.)[b] Count of survivors after exposure period, sec.		
	5	10	15	5	10	15
0.0	**0**	0	0	**51**	4	1
0.1	**0**	0	0	**71**	5	2
0.5	**0**	0	0	**129**	45	10
1.0	**48**	0	0	**176**	57	12
2.0	**19**	4	2	**135**	26	4
5.0	**130**	+	99	+	297	**54**
10.0	++	++	++	+	353	360
20.0	+++	+++	+++	++	+	+
50.0	++++	++++	++++	++++++	++++	+++

[a] *Germicidal solution contained 5,000,000 cells per ml. at start.*

[b] *Estimated number of cells in film on glass slide at start, 200,000: 99.9% destruction would leave 200 cells.*

carried out with both test organisms, using the glass slide technique. Concentrations of germicide were again selected to give approximately equal survival in the absence of added skim milk. The results (Table III) bring out several points. Against *S. aureus* Roccal at 100 p.p.m. was as effective as 200 p.p.m. of Dalglish, while in addition it retained its activity in the presence of far higher concentrations of skim milk. On the other hand, against *E. coli*, in the absence of added organic matter, 25 p.p.m. of Dalglish was as effective as 200 p.p.m. of Roccal. These findings concerning the relative potency of the two types of sanitizing agent in the absence of added organic matter agree with those recently published (9).

When the above concentrations of Roccal and Dalglish were compared against *E. coli* in the presence of more than 0.1% of added skim milk, Roccal appeared to better advantage. However, when further tests were run using 200 p.p.m. concentrations of both germicides, Dalglish was less sensitive to added skim milk than was Roccal (Table IV). Further tests with Dalglish showed good killing with 2% added skim milk, but with 5%, plates were uncountable.

Tests Using Tap Water

Mention was made earlier of the influence of the type of water used in preparing working dilutions of these germicides upon their activity. At this stage it was found that against *E. coli*, 50 p.p.m. of Roccal prepared with distilled water was roughly equivalent to 100 p.p.m. prepared with Ottawa tap water or to 200 p.p.m. prepared with a well water with a total hardness of 162 p.p.m. as calcium carbonate. Against *S. aureus* there was little difference. In the light of these findings, it was decided to repeat some of the

TABLE III

EFFECT OF ADDED SKIM MILK ON GERMICIDAL EFFICIENCY OF DISTILLED WATER SOLUTIONS OF ROCCAL AND DALGLISH. GLASS SLIDE TECHNIQUE

S. aureus[a] % added skim milk	Dalglish, 200 p.p.m. Count of survivors after exposure period, sec.			Roccal, 100 p.p.m. Count of survivors after exposure period, sec.		
	5	10	15	5	10	15
0.0	**8**	0	0	**6**	3	1
0.1	**11**	1	1	**2**	5	0
0.5	118	**7**	2	**67**	12	5
1.0	2800	1000	135	**86**	22	1
2.0	+++	+++	+++	160	**68**	23
5.0	++++	++++	++++	260	**96**	63
10.0	++++	++++	++++	458	238	**80**
20.0	++++	++++	++++	+	530	329
E. coli[b] % added skim milk	Dalglish, 25 p.p.m. Count of survivors after exposure period, sec.			Roccal, 200 p.p.m. Count of survivors after exposure period, sec.		
	5	10	15	5	10	15
0.0	**14**	3	5	**37**	3	2
0.1	**162**	9	3	**94**	5	3
0.2	1700	**111**	15	**155**	5	2
0.5	+++	+++	3000	1600	**9**	6
1.0	+++	+++	+++	++	2400	**121**
2.0	++++	++++	++++	+++	++	2100

[a] *99.9% destruction leaves 105 colonies per slide.*
[b] " " " *230* " " "

TABLE IV

EFFECT OF ADDED SKIM MILK UPON GERMICIDAL EFFICIENCY OF DISTILLED WATER SOLUTIONS OF ROCCAL AND DALGLISH. GLASS SLIDE TECHNIQUE. *E. coli*

% added skim milk	Dalglish, 200 p.p.m. Count of survivors after exposure period, sec.			Roccal, 200 p.p.m. Count of survivors after exposure period, sec.		
	5	10	15	5	10	15
0.0	**13**	9	4	**47**	30	12
0.1	**15**	13	4	**43**	17	16
0.2	**12**	12	7	264	**38**	38
0.5	**37**	43	27	+	**29**	17
1.0	**20**	21	18	++	+	278

N.B. *99.9% destruction leaves 190 colonies per slide.*

TABLE V

EFFECT OF ADDED SKIM MILK WHEN SOLUTIONS PREPARED WITH TAP WATER. ALL SOLUTIONS 100 P.P.M.

% added skim milk	*S. aureus*[a]						*E. coli*[b]					
	Count of survivors after exposure period, sec.						Count of survivors after exposure period, sec.					
	5	10	15	5	10	15	5	10	15	5	10	15
	Dalglish			Roccal			Dalgish			Roccal		
0.0	**8**	1	2	493	**53**	0	**10**	5	2	++++	++++	++++
0.1	**8**	1	1	448	**8**	2	**17**	4	3	++++	++++	++++
0.2	**9**	5	2	1320	**38**	7	**65**	7	4	++++	++++	++++
0.5	+++	+	**214**	+	**212**	8	615	**248**	214	++++	++++	++++
1.0	+++	+++	+++	++	+	**91**	+++	+++	+++	++++	++++	++++
2.0	+++	+++	+++	++	+	758	+++	+++	+++	++++	++++	++++
5.0				+++	+	857	+++	+++	+++	++++	++++	++++
10.0				+++	++	+						
20.0				+++	++	++						

[a] *99.9% destruction leaves 232 colonies.*
[b] " " " *310* "

above work using solutions prepared from Ottawa tap water, which showed a hardness of 64 p.p.m. In the first set of tests, both test organisms were compared against tap water solutions of Roccal and Dalglish containing 100 p.p.m. respectively of active ingredient. Table V shows that a Roccal solution prepared from tap water destroyed *S. aureus* in the absence of organic matter nearly as quickly as a solution prepared from distilled water (Table III). However, with larger addenda of skim milk, the tap water solution was definitely less active. The tap water solution of Dalglish compared more favorably with the Roccal solution than when distilled water solutions were compared (Table III), although again beyond a certain concentration of added skim milk there was a more abrupt drop in activity than with Roccal.

Turning now to *E. coli*, a much greater difference in the behavior of the two germicides was noted than with the distilled water solutions (Table IV). All plates from Roccal-treated slides were uncountable, while Dalglish showed excellent germicidal activity with additions of up to 0.2% skim milk. Beyond 0.5%, however, the solutions were ineffective.

Since Roccal at 100 p.p.m. proved so ineffective against *E. coli*, similar tests were conducted using 200 p.p.m. solutions of each germicide. In addition, a further change was made. In previous studies, the required amount of skim milk had been added to all lots of germicidal solution before starting to measure the germicidal activity of the first solution. Subsequently, titrations of Dalglish solutions shortly after they had been used to treat the glass slides indicated a marked decline in strength with the higher concentrations of added skim milk. Consequently, in order to place all solutions on a more even footing in respect to initial strength, the procedure was

changed and the skim milk added to the test solution immediately before introducing the first glass slide for germicidal treatment. The results from these tests appear in Tables VI and VII. With *E. coli*, in order to be able to measure the effect of the added skim milk on the Roccal solution, longer exposure periods were employed with Roccal than with Dalglish. Surprisingly enough, the change in the time of adding the skim milk had less effect with Dalglish than was expected.

TABLE VI

EFFECT OF ADDING SKIM MILK IMMEDIATELY BEFORE TESTING WHEN SOLUTIONS PREPARED WITH TAP WATER. *E. coli*

% added skim milk	Dalglish, 200 p.p.m. Count of survivors after exposure period, sec.			Roccal, 200 p.p.m. Count of survivors after exposure period, sec.		
	5	10	15	10	20	30
0.0	**4**	1	3	++	564	**39**
0.1	**11**	10	4	++	**213**	40
0.2	**4**	4	3	++	**206**	112
0.5	**5**	3	1	++	+	392
1.0	**6**	4	1	+++	+++	++
2.0	**48**	123	199	+++	+++	++
5.0	++	+++	+++			
Solutions prepared 24 hr. before testing, then reinforced						
0.1	**12**	5	2	840	**13**	6
0.2	**1**	5	2	+	**21**	13

N.B. *99.9% destruction leaves 310 colonies per slide.*

While these results indicate that such concentrations of skim milk as might be picked up by sanitizing rinses do not interfere with the germicidal activity, it was deemed advisable to see whether this also held true where the solutions containing added skim milk were held for 24 hr. before testing. This would more nearly approximate the condition of a sanitizing rinse recovered after use and re-used the following day. Consequently, small amounts of skim milk were added to solutions of Roccal and Dalglish, then after 24 hr. at room temperature the strength of Dalglish was readjusted to the original concentration. (Roccal solutions showed no significant drop in strength when tested with the Universal Test Kit*, so they were not reinforced.) The results of one such test, shown in the lower part of Table VI, indicate no reduction in potency of Dalglish solution, while Roccal actually shows enhanced activity against *E. coli*. Results from similar tests with *S. aureus* appear in the lower section of Table VII. Here, instead of comparing the reinforced solutions of Dalglish against the freshly prepared solution of full strength, comparisons

* *Obtained from Dr. Geo. J. Hucker, Geneva, N.Y.*

TABLE VII

EFFECT OF ADDING SKIM MILK IMMEDIATELY BEFORE TESTING WHEN SOLUTIONS PREPARED WITH TAP WATER. *S. aureus*

% added skim milk	Dalglish, 200 p.p.m. Count of survivors after exposure period, sec.			Roccal, 200 p.p.m. Count of survivors after exposure period, sec.		
	5	10	15	5	10	15
0.0	**3**	1	1	**49**	2	1
0.1	**1**	2	1	**47**	1	0
0.2	**4**	0	1	**35**	1	0
0.5	**3**	2	1	**61**	0	0
1.0	**21**	2	1	**129**	8	0
2.0	**17**	8	4	570	413	**94**
5.0	+++	+++	+++	+	+	**163**
10.0				++	+	386
20.0				+++	++	637
Solutions prepared 24 hr. before testing, not reinforced						
0.1[a]	**2**	3	0	**32**	1	0
0.2[b]	**3**	1	2	**34**	2	1
Solutions freshly prepared to match strength of 24 hr. old solutions						
0.0[a]	**61**	5	3			
0.0[b]	**26**	9	4			

[a] *Dalglish solution 108 p.p.m.*
[b] " " *42* "

N.B. *99.9% destruction leaves 385 colonies per slide.*

were made between the unreinforced solutions and freshly prepared solutions of the same chlorine content. The results again indicate no reduction in the potency of either sanitizing agent as a result of adding skim milk. Similar results were obtained starting with 100 p.p.m. solutions of both germicides, and using either whole or skim milk addenda.

In an endeavor to discover the reason for the discrepancy between these results and those of Mallmann and Edwards (11), an attempt was made to duplicate their experiment. However, when a tap water solution of Dalglish containing 20 p.p.m. available chlorine plus 0.2% skim milk was held for 24 hr. at room temperature in the dark, the chlorine was entirely dissipated. The greater loss here than that reported by the above workers was doubtless due to the higher pH value of our solution (9.88 for 100 p.p.m.) compared with their chlorine water solution. As Wright (16) and Prucha (13) have shown, increased alkalinity hastens the loss of chlorine in the presence of organic matter.

Further tests were run using Dalglish tap and distilled water solutions of varying original strengths, held for 24 hr. after adding skim milk, comparing against freshly prepared solutions of equivalent available chlorine concentration. The results appear in Tables VIII and IX. They show that a 'used' solution titrating 12 or 13 p.p.m. available chlorine was far less active than a freshly prepared one showing the same strength by titration; at 30 to 35 p.p.m., the difference was slight, and at 100 p.p.m. it had disappeared.

TABLE VIII

GERMICIDAL EFFICIENCY OF 'USED' HYPOCHLORITE SOLUTIONS VS. *S. aureus*. GLASS SLIDE TECHNIQUE

Solution	% added skim milk	P.p.m. Av. Cl		Count of survivors after exposure period, sec.					
		As prepared	As used	2	5	10	20	40	60
A[a]	0.1	40	12		++++	+++	+++	++	+
B[a]	0.0	13	13		**210**	32	33	9	7
A[a]	0.1	50	12.5		++++	+++	+++	++	+
B[a]	0.0	12.5	12.5		**19**	7	3	6	1
C[b]	0.1	50	7.3		++++	++++	+++	+++	++
A[b]	0.1	100	34	++++	+++	**146**			
B[b]	0.0	32	32	++++	+++	**32**			
C[b]	0.1[c]	100	30	++++	+++	**41**			
D[b]	0.0	50	50	455	**13**	9			
E[b]	0.1	50	50	1150	**28**	5			
F[b]	0.2	50	50	++	**160**	16			
A[b]	0.1	200	108	**49**	6	3			
B[b]	0.0	105	105	**45**	11	4			
C[b]	0.2	200	38	+++	**89**	27			
D[b]	0.0	194	194	**23**	1	1			

[a] *Prepared with distilled water.*
[b] " " *tap* "
[c] *Whole milk addendum.*
Solutions A and C held with added skim milk for 24 hr. at room temperature before testing; remainder prepared immediately prior to testing.

Practical Tests with 'Used' Solutions

To determine whether the slight potentiating action of small addenda of skim milk observed with the glass slide technique would show up under practical conditions, tests were made using two small (50 gal.) coil pasteurizing vats. Various procedures were tried before the following was adopted. The vats were sterilized with steam, then after cooling sprayed with skim milk containing between two and five million cells of the test organism per milliliter. The vats were allowed to stand overnight with the lids closed, so that the film of skim milk would dry onto the walls. Next morning an area of 1 sq. ft. on each vat was swabbed with a moist cotton swab, the stick cut,

TABLE IX

GERMICIDAL EFFICIENCY OF 'USED' HYPOCHLORITE SOLUTIONS VS. *E. coli*. GLASS SLIDE TECHNIQUE

Solution	% added skim milk	P.p.m. Av. Cl		Count of survivors after exposure period, sec.					
		As prepared	As used	2	5	10	20	40	60
A[a]	0.1	40	12		++++	+++	+++	++	+
B[a]	0.0	13	13		**30**	24	19	16	8
A[a]	0.1	50	12.5		++++	+++	+++	++	++
B[a]	0.0	12.5	12.5		**25**	9	25	2	3
A[b]	0.1	100	34	++	**65**	6			
B[b]	0.0	34.5	34.5	**111**	10	7			
C[b]	0.1[c]	100	35	**65**	9	5			
D[b]	0.0	50	50	**15**	7	3			
E[b]	0.1	50	50	**44**	27	5			
F[b]	0.2	50	50	107	**19**	4			
A[b]	0.1	200	108	**5**	2	3			
B[b]	0.0	105	105	**6**	2	2			
C[b]	0.2	200	38	192	**16**	5			
D[b]	0.0	194	194	**12**	3	2			

[a] *Prepared with distilled water.*
[b] " " *tap* "
[c] *Whole milk addendum.*

Solutions A and C held with added skim milk for 24 hr. at room temperature before testing; remainder prepared immediately prior to testing.

and the cotton dropped into a dilution blank containing 99 ml. of sterile physiological saline. After shaking for one minute, plates were prepared and incubated at 32° C. for 48 hr.

One vat was then sprayed with Dalglish solution made up to 100 or 200 p.p.m. available chlorine the previous day, at which time 0.1% of skim milk had been added. After holding overnight in the dark at room temperature this solution was reinforced to its original strength, while a solution freshly prepared to match the titratable chlorine content was used to spray the other vat. A different area was swabbed immediately after the spraying, and a third area two hours later to determine the residual effect. Plates were prepared from each swab the same as for the control swabs. *S. aureus* and *E. coli* were again used as test organisms.

The results from several tests are given in Table X. Here there is no evidence of a reduction in sterilizing effectiveness where the 'used' hypochlorite solution, which had stood overnight at room temperature, was reinforced to its original strength. On the contrary, in every instance the 'used' solution was more effective than the fresh. Nor did the fact that the organisms suspended in skim milk had been sprayed on the vat walls appear to have had any effect. (In the earlier tests, suspensions of the test organism in 10%

TABLE X

EFFECTIVENESS OF FRESH VS. 'USED' HYPOCHLORITE SOLUTIONS IN SANITIZING PASTEURIZING VATS

	Available chlorine, p.p.m.			Control area plate count per square foot[a]	Treated area, % survival after spraying			
		'Used'			Swabbed immediately		Swabbed after 2 hrs.	
	Freshly prepared	Before reinforcement	After reinforcement		A[b]	B[b]	A[b]	B[b]
S. aureus	200	125	200	9,200[c]	—	2.0	7.2	5.5
	99	34	96	290,000	4.4	0.34	0.82	0.06
	100	32	100	1,500,000	1.56	0.0004	<0.016	<0.0004
E. coli	205	116	198	350,000	0.16	0.05	0.32	0.09
	97	12	100	2,300,000	0.87	0.23	1.22	0.48

[a] *Average of swabbed areas of both vats.*

[b] *A—Solutions freshly prepared. B—Solutions containing 0.1% skim milk prepared the previous day and reinforced to original strength before using.*

[c] *Vats hosed down with cold water two hours after spraying with skim milk suspension of test organism.*

skim milk were used; in addition, the vats were rinsed down with clear cold water two hours after being sprayed with the test organisms. However, where this was done, the number of organisms present after the sanitizing treatment was so small that satisfactory counts could not be obtained). Despite the fact that the test organisms were present in the skim milk layer on the walls of the vat, the percentage reduction in count as a result of spraying with the hypochlorite solutions was very gratifying. The data also suggest a continuing germicidal activity against *S. aureus* on the part of the residual hypochlorite during the two hour period subsequent to the sanitizing treatment.

Discussion

An interesting feature of these studies has been the finding that small concentrations of skim milk, such as might be picked up by a sanitizing rinse, not only fail to depress the germicidal activity of the two germicides employed, but frequently cause a slight enhancement of activity against both test organisms. This is decidedly at variance with the popular concept of the effect of added organic matter upon germicidal activity, particularly with hypochlorites, and is in marked contrast to the results reported by Mallmann and Edwards (11).

The effect of organic matter upon the germicidal activity of hypochlorite solutions appears to depend very largely upon the strength of the solution at the time of use. As will be seen from the data in Tables VIII and IX a 'used' hypochlorite solution titrating 12 or 13 p.p.m. available chlorine had far less germicidal power than a fresh one showing the same strength by titration. At 30 to 35 p.p.m. the differences were slight, while at 100 p.p.m. they had disappeared. These results help resolve the contradiction between

the findings of Mallmann and Edwards and our own. Had they started with solutions of the strengths commonly used in milk plants (50 to 200 p.p.m. available chlorine), they would doubtless have reached different conclusions concerning the germicidal activity of 'used' hypochlorite solutions.

The importance of thoroughly cleaning the surfaces of food handling equipment before attempting a sanitizing treatment has been universally stressed. Even though our results fail to show the expected reduction in germicidal activity of hypochlorites and quaternary ammonium compounds in the presence of added skim or whole milk, it cannot be overemphasized that this must not be interpreted as meaning that thorough cleaning of equipment is unimportant. Where milk or other food residue is allowed to remain, unsightly films build up which interfere with heat transfer while protecting resistant types of bacteria. The large numbers of thermoduric organisms found in milk that has been handled in milking utensils coated with milkstone (a sure indication of faulty cleaning procedure) are an indication of what may be expected where cleaning is inadequate.

The slight potentiating effect of small addenda of skim milk upon the germicidal activity of Roccal and Dalglish solutions is in line with our findings previously reported (9) that skim milk increased the bacteriostatic and bactericidal effect of quaternary ammonium compounds in the glass slide technique. However, the explanation advanced at that time (absorption and carry-over of larger amounts of the germicide on the film of milk) is inadequate in this case. Wolf and Cousins (15), discussing their findings that vegetative cells dried in a milk film on a metal surface show optimum 'kills' at much higher pH values than was expected, postulate a reaction between the hypochlorite and the protein film to form a 'chloroamine', which is more germicidal than hypochlorite at pH values above 9.5. Even assuming that a similar reaction takes place with the glass slide technique, this fails to explain why the presence of additional small amounts of skim milk in the germicidal solution itself appears to increase its activity.

The influence of the type of water used in diluting the germicides on the susceptibility of the two test organisms was quite striking. (Further studies on this phase of the work will be reported separately.) While Roccal solution prepared with distilled water was appreciably more effective against *S. aureus* than against *E. coli*, the difference was slight compared to that shown by solutions prepared with tap water. Since the quaternary ammonium compounds usually become more effective with increasing pH (2, 5, 6, 9), while here the reverse holds true, some factor other than pH is evidently involved. The hypochlorite, on the other hand, was slightly more effective in tap water solution, presumably on account of the lower pH of the solution (4, 7) resulting from the buffering action of the tap water.

Both germicides retained a surprisingly high degree of effectiveness in the presence of considerable concentrations of skim milk. Roccal showed a gradual decline in effectiveness with increasing amounts of organic matter, whereas the hypochlorite showed a sudden drop.

It will be observed that the plating technique shows a much greater reduction in bacterial numbers than does the glass slide technique. That organisms freely suspended in the germicide solution would be more easily killed than those present on a surface in a film of skim milk is to be expected. In addition, the clumping of bacteria in quaternary ammonium solutions, as reported by McCulloch (12), may be a factor in lowering the plate count of survivors. A technique in which the organisms are present on a surface is therefore likely to yield results more representative of those obtained in the actual sanitizing of surfaces of food handling equipment.

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Addendum

Since this paper was prepared, Neave and Hoy (J. Dairy Res. 15 : 24-54. 1947.) have reported that the addition of 0.2% of whole milk to a hypochlorite solution containing 200 p.p.m. available chlorine had no significant effect upon the destruction of *S. aureus*. Their statement that "If the milk had remained in contact for a longer period and more tests had been made, there is no doubt that a significant decrease in germicidal power would have been shown" does not appear to have been substantiated by our results. They also refer to the work of Bryan *et al.* (J. Milk Tech. 5 : 77-85. 1942.), which indicated that the addition of 5% milk to hypochlorite solutions containing 40 to 200 p.p.m. available chlorine greatly lowered the germicidal efficiency against mastitis streptococci.

CHEMICAL AND MICROBIOLOGICAL STUDIES ON STORED, SALTED BUTTER[1]

By G. A. Grant,[2] N. E. Gibbons[2], J. B. Marshall,[2] and H. J. Lips[2]

Abstract

Salted butter from two Canadian plants was stored in parchment wrappers and in cans at −1.1°, 10.1°, 21.1°, and 32.2° C. (30°, 50°, 70°, and 90° F.). Deterioration of the stored samples was followed by assessment of flavor, and by determination of: total acidity, free acidity, amino acid, pH, and fluorescence of the butter serum; peroxide oxygen, free fatty acids, and fluorescence of the butterfat; acidity of the whole butter; the number of viable, proteolytic, lipolytic, and oxidase positive organisms; and yeast and mold counts.

Butter in cans deteriorated less rapidly than print samples although differences were less pronounced at 70° and 90° F. than at 30° and 50° F. Canned samples did not change appreciably in quality during 45 weeks' storage at 30° F. In general, decomposition of the serum and flavor deterioration became evident before changes occurred in the fat fraction. Stability of the serum was the limiting factor in keeping quality. The formation of amino acids and intermediate products of protein decomposition contributed largely to flavor changes.

Flavor scores were more closely related to chemical objective test values than to microbiological counts. Storage temperature showed a marked influence on the correlation of objective test values with flavor score. Of the objective tests studied, total serum acidity (amino acidity plus free serum acidity) pH and serum fluorescence were most closely associated with flavor score.

Introduction

The production of butter is seasonal and is usually concentrated in areas where fluid milk is not readily marketed. These factors emphasize the importance of keeping quality if butter is to reach the consumer in good condition. Many studies have been made to improve methods of handling the cream and of packaging butter. It has been reported that butter develops tainted flavors when stored in wooden containers (8, 11, 18). Partial success in preventing the production of these flavors by the use of various types of liners has been reported (2, 3, 17).

Butter has been shipped to countries in the tropics for many years, often arriving in poor condition, and consumers there have become conditioned to using a product that is undesirable by temperate zone standards. This state of affairs is accepted as inevitable by consumers and exporters alike, and no particular effort has been made to improve the quality of canned butter for export. During the war, canned butter was included in Red Cross parcels and in special service rations. In an effort to extend the storage life of butter for these purposes, greater attention than heretofore was given to initial quality.

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The information available about keeping quality is contradictory and relates chiefly to studies of accelerated shelf tests. This investigation was carried out to obtain factual data on changes in butter at storage temperatures above those used in warehouses (i.e. −10° to 0° F.). As butter contains more than one component, it is logical that storage temperature may affect one component more than another and thus deterioration may be favored in specific fractions at different temperatures. Therefore, the butter was stored over a range of temperatures to try to establish the relative susceptibility of different components at high and low temperatures.

Many factors have been suggested as responsible for spoilage in butter, and it was felt that no one test would satisfactorily assess keeping quality. Although organoleptic tests measure the over-all quality of butter, they lack uniformity between investigators. Therefore, a number of chemical and microbiological determinations were carried out on whole butter, butter serum, and butterfat from stored whole butter.

The present study compares the keeping quality of canned and print butter over a wide range of temperatures using a number of chemical and microbiological methods. The degree of association between the results of these methods and organoleptic assessment was also determined.

Experimental

The experimental material consisted of two lots of commercial first grade Canadian salted butter prepared from pasteurized cream. One lot was selected from a single churning of Western butter and the second was prepared from selected neutralized cream in an Eastern creamery. Part of the latter lot was canned immediately in lacquered cans without parchment wrapping and an equal part prepared in parchment-wrapped 2-lb. prints. The Western butter churning was selected on the basis of low yeast and mold counts and an accelerated storage test. Cylindrical 1-lb. prints were then prepared and wrapped in parchment; half of these were sealed in lacquered cans. All the material was shipped by refrigerated car to Ottawa and held at −40° F. until allotted at random to experimental conditions.

The canned and the print butter from both plants were stored at 30°, 50°, 70°, and 90° F. The prints were stored in sterile beakers covered with brown wrapping paper. Samples were withdrawn at intervals depending on storage temperature: at 30° F., after 16, 32, 64, 128, 192, 256, 321, and 385 days; at 50° F., after 4, 8, 16, 32, 64, 96, 128, 192, and 256 days; at 70° F., after $\frac{1}{2}$, 1, 2, 3, 4, 8, 16, 32, 64, and 128 days; and at 90° F., after $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 3, 4, 8, 16, and 32 days.

Methods

A quantitative determination by chemical methods of the substances causing off-flavors in butter is difficult, because of the complexity of the reactions accompanying spoilage. However, chemical and microbiological methods are undoubtedly of value for comparison of similarly treated products.

The following objective measurements were selected for study: peroxide oxygen, free fatty acid, Kreis value, and fluorescence of the fat fraction; acidity of whole butter; amino acid by Sorensen's titration, and acidity and fluorescence of the butter serum. Viable, lipolytic, proteolytic, and oxidase positive organisms; and yeast and mold counts on the whole butter were also included.

Preparation of the Sample for Analysis

Samples for microbiological examination were taken first with sterile cork borers, made of stainless steel. The butter stored at 90° F. was very soft, necessitating cooling for a few hours at 45° F. When time did not permit hardening by cooling, the wrapper was removed, the sample mixed, and representative portions taken with a sterile tongue depressor. The square prints were sampled through the face opposite to that on which the parchment flaps met. With the round prints, borings were taken through the face just past the paper overlap. The cans were flamed on top and opened with a wheel-type can opener that had been flamed. The lid and cover paper were removed and borings taken at and around the center. Enough material was taken from the cans and prints to give at least 20-ml. portions when melted. Samples of whole butter were withdrawn for immediate organoleptic rating and whole butter acidity measurements.

Serum and fat were separated by a method previously described (10). Briefly, the butter is heated in centrifuge bottles placed on a steam bath at 212° F., stirred (with a thermometer) until it reaches 110° to 120° F., then centrifuged for 10 min. at 1700 r.p.m. The fat is removed by siphoning and filtering through absorbent cotton, and part of the clear portion of the serum is pipetted into a separate container.

Organoleptic Ratings

Two sets of organoleptic ratings were obtained. The butter was scored for flavor only by three professional graders, by methods outlined in the Canadian Dairy Industry Act and Regulations, 1942, pp. 48 to 51. The second system, which permitted statistical computations, employed a panel of 10 judges to assess flavor as follows: 10, excellent; 8, good; 6, fair; 4, poor; 2, bad; 0, unapproachable. At any one time the 10 tasters were required to score a set of four samples chilled to approximately 50° F.

Acids

The free fatty acids of the butter fat were determined by titrating 10 gm. dissolved in 50 ml. of hot ethanol with 0.02*N* sodium hydroxide, until neutral to phenolphthalein. This procedure was also used to determine acidity of the whole butter, which includes other titratable acids formed from protein and carbohydrate breakdown. A colorimetric method (13) was tried but proved to be inadequate.

A difference in amino nitrogen, as determined by Van Slyke's method (19, 20), was obtained between serums of fresh and spoiled butter, (0.073 mgm. of amino nitrogen per ml. of serum for fresh butter, 0.159 mgm. for

spoiled butter). This finding with the evidence of other investigators (15) suggested that protein deterioration was taking place. The acidity of the serum due to free acids, such as lactic, was determined by titrating an aliquot with 0.02 *N* sodium hydroxide, employing phenolphthalein as an indicator. The values are reported as mgm. of sodium hydroxide per 100 ml. of serum, and are termed free serum acidity. To obtain an estimate of the protein decomposition, the formation of amino acids was followed by Sorensen's titration. Although this method has its limitations, it enabled comparison between samples. The values are reported as mgm. of sodium hydroxide per 100 ml. of serum and are termed amino acids.

To estimate the over-all change in the serum, the values for free serum acidity and amino acids were combined and termed total serum acidity.

The hydrogen ion concentration of the serum was determined at 77° F. on 10 ml. of the serum by means of a pH meter.

Fluorescence

Since many types of fat show changes in fluorescence with storage (16), it was considered desirable to examine the change in the fat from stored butter. One-gram samples of butterfat from fresh and spoiled butter were dissolved in 10 ml. portions of solvents and their fluorescence measured on a Coleman photofluorometer with filters transmitting light in the region of 365 mμ. The fluorescence readings are given in Table I. Of the solvents used, benzene

TABLE I

FLUORESCENCE OF BUTTERFAT DISSOLVED IN ORGANIC SOLVENTS

Solvent	Fresh butter	Spoiled butter	Difference
Benzene	64.5	41.0	23.5
Xylene	58.5	37.0	17.5
Dioxane	51.5	38.0	13.5
Ethyl ether	30.0	22.5	7.5
Ethylene dichloride	37.5	31.0	6.5
Amyl acetate	32.0	26.0	6.0
Petroleum ether	39.5	34.0	5.5
Acetone	15.5	17.5	2.0
Chloroform	21.5	20.0	1.5
Carbon tetrachloride	6.5	8.0	1.5
Ethanol	Too turbid	—	—
Methanol	Too turbid	—	—

and xylene resulted in the largest difference between fresh and rancid fat. Xylene was selected for further study. The effect of fat concentration was also investigated. The results in Table II indicate that the fluorescence of butterfat from fresh and spoiled butter increased with fat concentration, and also that the differences between fresh and spoiled butter were larger at the higher concentrations. As the differences between the spoiled and fresh butter were not the same at all concentrations tested, a standard concentration of

10% butter fat was chosen. However, as the test was not totally satisfactory, it was applied only to a limited number of samples. Serum fluorescence was determined as described previously (10).

TABLE II

THE EFFECT OF BUTTERFAT CONCENTRATION IN XYLENE ON FLUORESCENCE VALUES

Percentage of butterfat	Fresh	Spoiled	Difference
10	62.0	45.0	17.0
20	85.5	64.0	21.5
30	96.0	74.0	22.0

Peroxide Oxygen

Both the ferrometric and iodometric methods (5, 7) were employed for the estimation of peroxide oxygen value, for reasons outlined in a recent investigation on lard (9).

Kreis test

The modification by Walters *et al.* (21) of the original Kreis test (14) was selected as the most suitable because of its simplicity and development of color in a single phase system. Investigation by White (22) showed that the color intensity was proportional to the concentration of fat. One gram of butter oil was employed in each test. If the intensity of color after the reaction was too great, a dilution with solvent was made. The Evelyn photoelectric colorimeter (6) provided with a 580 Rubicon filter was found to be quite satisfactory for measurement of color intensity. The results are reported as modified extinction coefficients. Although the values obtained have little theoretical significance, they are quite suitable for comparison between samples.

Microbiological Methods

Samples were held at 40° F. until they could be plated. They were then melted at 110° F. and 10 ml. pipetted into a 90 ml. water blank preheated to 110° F. Appropriate dilutions were made in the blanks.

Viable counts were made on tryptone glucose agar with 0.5% skim milk added (1, p. 22). Both proteolytic (caseinolytic) and viable organisms were estimated on these plates, counts being made at two and three days after incubation at 90° F. and three and five days at 70° F. Yeasts and molds were estimated on Difco potato dextrose agar adjusted to pH 3.5 to 4.0 just before use. Counts were made after three and five days at 70° F. For the estimation of lipolytic organisms, butterfat was prepared from butter having a low free fatty acid content and no iodometric peroxide oxygen value and stained with neutral red base (12). Five per cent of stained fat was added to 2% agar containing 0.5% each of Difco proteose–peptone and tryptone.

Only colonies imparting a distinct red color to the fat globules under or around them were considered lipolytic. Oxidase positive organisms were detected on the same medium by using unstained fat and flooding the plates with a weak solution of dimethylparaphenylenediamine. Both lipolytic and oxidase positive organisms were counted after five days at 90° F. and seven days at 70° F.

Treatment of Data

The many data required statistical analyses to assess the relative importance of the factors involved. Each test is reported first from the standpoint of treatment effects. These effects are shown in detail in graphical form in Figs. 1 to 4, in which the results from the two plants are averaged, as it was more desirable to emphasize differences due to temperature and packaging than those due to source. The relation of chemical tests to flavor, and their assessment of keeping quality, are indicated by correlation and regression coefficients and an analysis of covariance (Tables IV to VI).

Results

Flavor

The mean flavor scores as assessed by the laboratory flavor panel are shown in Fig. 1. An increase in storage temperature or time caused a decrease in flavor score. The flavor score of the print butter decreased more rapidly than did that of canned butter at all the storage temperatures. There was only a slight decrease in flavor score of the canned butter after storage for 45 weeks at 30° F. At 50° F., the canned butter decreased in the first 14 weeks, then showed a definite improvement.

Further details of flavor results obtained by the butter graders and laboratory flavor panel are given in Appendix Tables I and II. For the purpose of this investigation the laboratory flavor assessment system was superior to that employed commercially as it could be correlated more easily with objective tests and furnished a more reliable assessment of the butter flavor by employing a larger group of tasters and a scoring system with equal increments.

Fluorescence

An increase in storage time or storage temperature resulted in an increased serum fluorescence value (Fig. 1). The rate of increase in the fluorescence was greater at 70° and 90° F. than at 30° and 50° F. The print butter showed a greater increase in serum fluorescence values at all the storage temperatures than that stored in cans. The canned butter stored at 30° and 50° F. showed approximately six units increase in serum fluorescence in 45 weeks.

As the fluorescence determinations on the fat fraction were not very informative, only a few examples are given in Table III. The fluorescence of the fat fraction did not change appreciably with flavor deterioration at any of the storage temperatures.

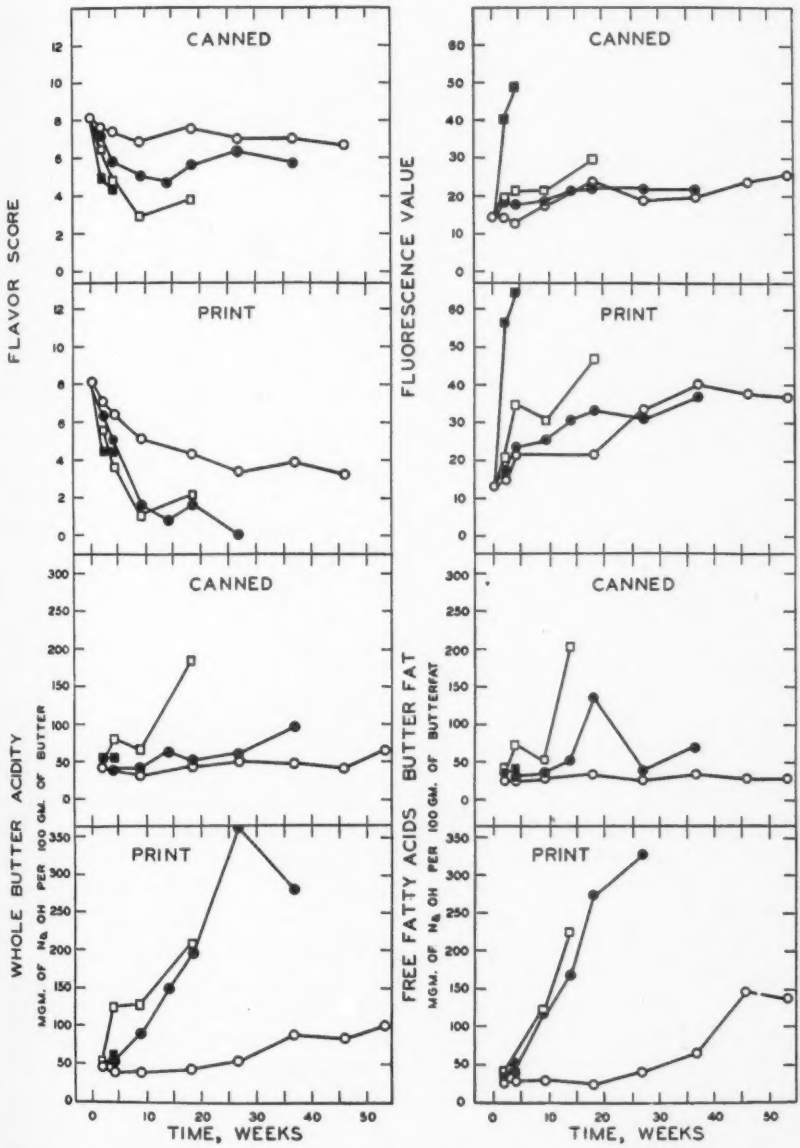

FIG. 1. *Effects of storage at 30°, 50°, 70°, and 90° F. on the laboratory flavor panel score, serum fluorescence, whole butter acidity, and the free fatty acid content of butterfat from canned and print, salted butter.* 30° F., ○; 50° F., ●; 70° F., □; 90° F., ■.

TABLE III

FLAVOR SCORES AND CHEMICAL MEASUREMENTS ON THE FAT FRACTION FROM BUTTER STORED AT 30°, 50°, 70°, AND 90° F.

(Values averaged over both plants and both packages)

Storage Temperature, °F.	Flavor score	Peroxide oxygen content		Kreis value	Fluorescence value
		Ferrometric	Iodometric		
90	8.1	0.0	0	0.3	60
	4.7	3.8	0	0.6	57
	4.4	1.7	0	0.7	56
70	8.1	0.0	0	0.3	60
	6.0	3.7	0	0.5	55
	4.2	1.5	0	0.6	57
	2.0	2.0	0	2.0	56
50	8.1	0.0	0	0.3	60
	6.8	2.8	0	0.4	56
	5.4	1.3	0	0.5	58
	3.3	1.6	0	0.3	56
	3.2	2.2	0.3	8.2	58
	2.8	5.1	1.3	6.5	—
30	8.1	0.0	0	0.3	60
	7.3	2.7	0	0.4	—
	6.8	1.8	0	0.4	55
	7.5	1.4	0	—	58
	7.0	2.5	0	—	56
	6.8	1.6	0	4.0	—
	5.8	—	0	—	—

Acids

The acidity determinations on the whole butter generally showed an increase with storage time and storage temperature for both canned and print wrapped butter (Fig. 1). The increase was more pronounced for print than for canned butter. The formation of acidity was suppressed in the canned butter stored at 30° and 50° F., and as this measurement is some indication of the hydrolysis of protein and fat, it is inferred that canning the material retarded the rate of spoilage. The canned butter showed only a slight increase in acidity after storage for 45 weeks at 30° F.

The free fatty acid content of the butter fat increased with storage time and storage temperature for both canned and print material (Fig. 1). There was a more prominent increase in the free fatty acids in the butter stored in prints than that stored in cans. There was little change in free fatty acids in canned material even after storage at 30° F. for 45 weeks.

From Fig. 2 it may be seen that the free serum acidity increased with storage time, the rate varying with the storage temperature. The butter stored in prints showed a greater rate of increase at all the storage temperatures. The butter stored at 30° F. in cans showed very little increase after 45 weeks, while that stored in the prints showed a substantial increase after 20 weeks.

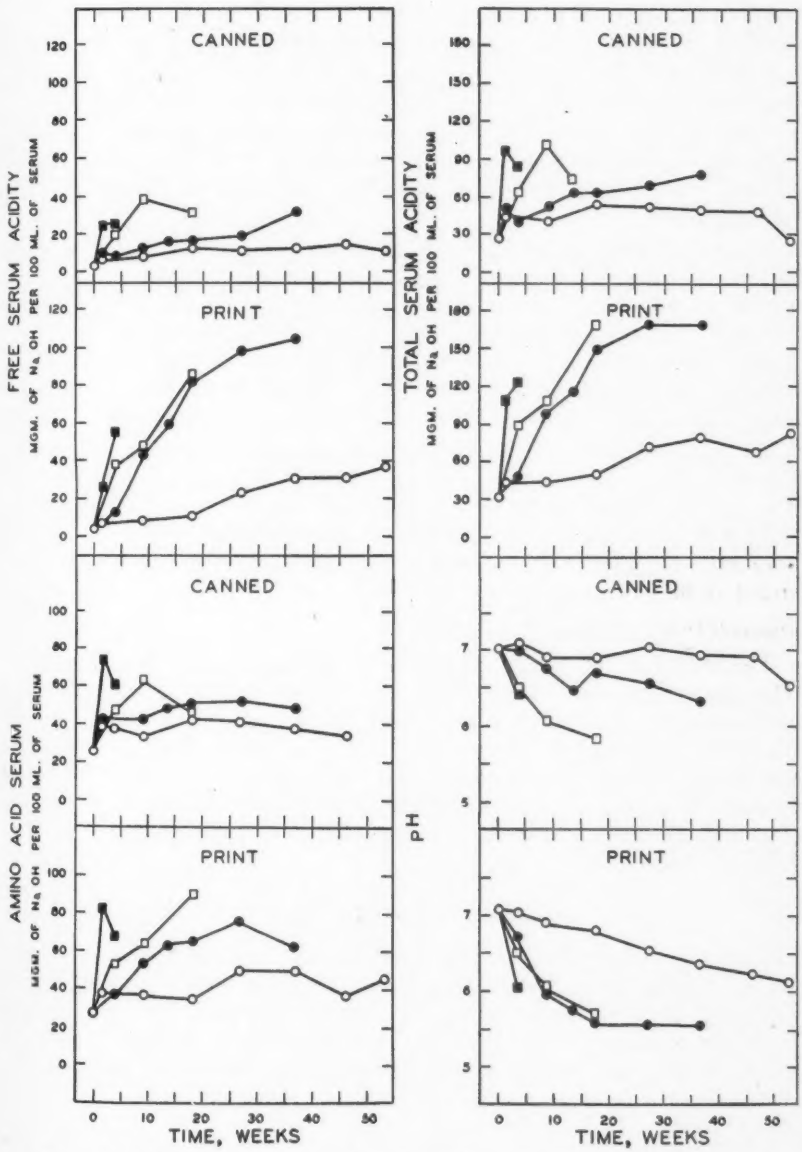
CANNED
PRINT
FREE SERUM ACIDITY
MGM. OF Na OH PER 100 ML. OF SERUM
TOTAL SERUM ACIDITY
MGM. OF Na OH PER 100 ML. OF SERUM
AMINO ACID SERUM
MGM. OF Na OH PER 100 ML. OF SERUM
pH
TIME, WEEKS

FIG. 2. *Effects of storage at 30°, 50°, 70°, and 90° F. on free serum acidity, amino acid in the serum, total serum acidity, and pH of the serum from canned and print, salted butter. 30° F., ○; 50° F., ●; 70° F., □; 90° F., ■.*

Canning of the butter appeared to suppress the formation of free serum acidity most at 30° and 50° F.

The amino acids showed an increase followed by a decrease with storage time (Fig. 2). This was accentuated at the higher storage temperatures. The rate of increase in the amino acids was greater at 70° and 90° F. than at 30° and 50° F. At all the storage temperatures the amino acid content developed somewhat more rapidly in the print butter than in the canned butter. The canned material stored at 30° F. showed little change after the first two weeks.

Increases in total serum acidity with period of treatment for both canned and print butter at all the storage temperatures may be seen from Fig. 2. In some cases, there was first an increase, then a decrease after prolonged storage. This was quite evident in butter stored in cans at 70° and 90° F. At all storage temperatures, print butter showed larger increases in total serum acidity than canned butter. From this evidence, it is apparent that the over-all changes in the serum were greater for material stored in prints.

The hydrogen ion concentration of the butter serum decreased with storage time (Fig. 2), the decrease being more rapid at the higher storage temperatures. The changes in print butter were greater than those in canned material. As the pH is a function of the fermentation taking place, it is considered that very little deterioration from this source took place in the canned butter stored at 30° F. for 45 weeks.

Peroxide Oxygen Content, Kreis Value

The results of determinations on the fat fraction are shown in Table III. The peroxide content as measured by both methods showed no large increase with flavor deterioration at any of the storage temperatures. The aldehyde content as measured by Kreis value showed a slight increase with flavor deterioration at the lower temperatures. The lack of appreciable increases in peroxide oxygen content and aldehyde with flavor deterioration suggests that oxidative changes in the fat fraction contribute little to the flavor deterioration in stored salted butter.

Microbiological Tests

Since there was little difference in the counts after incubation at 70° and 90° F., averages were taken, and since differences between conditions of storage were of primary interest, the results for butter from both sources were also averaged for graphical presentation. Owing to sampling errors, the bacteriological estimations show considerable variation. However, the data presented in Figs. 3 and 4 show the trends found at various storage temperatures.

The original number of both the viable and proteolytic organisms was slightly greater in the canned than in the print butter. In the canned material, there was a small but rapid increase during the first two days at 70° and 90° F. The general trends with all types of organisms were very similar in butter

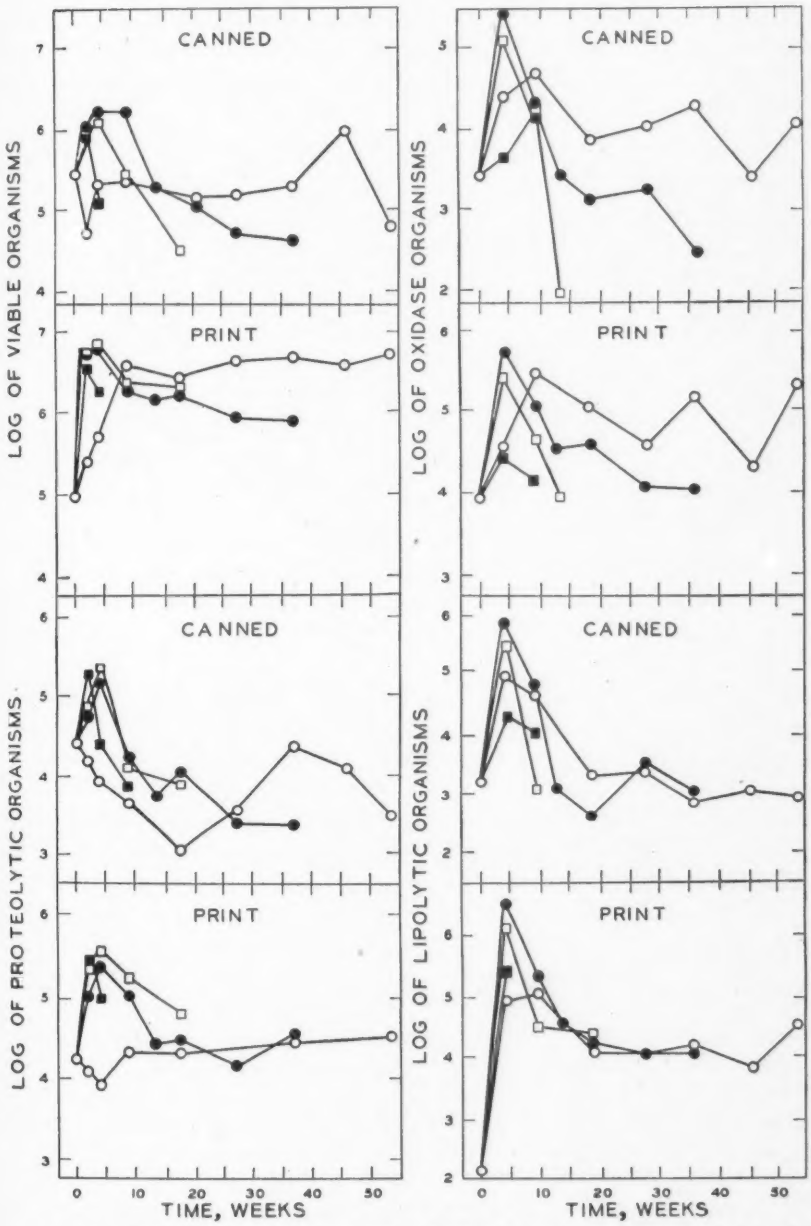

FIG. 3. *Effects of storage at 30°, 50°, 70°, and 90°, F. on viable, proteolytic, lipolytic, and oxidase positive organism contents of canned and print, salted butter. 30° F., ○; 50° F., ●; 70° F., □; 90° F., ■.*

stored at 90° F. The number of bacteria reached a peak in three to four days, remained at approximately that level for 8 to 16 days, and then decreased. At 70° F., the peak came in four to eight days and the numbers remained fairly constant for 32 days. At 50° F. the increase was slower, the numbers

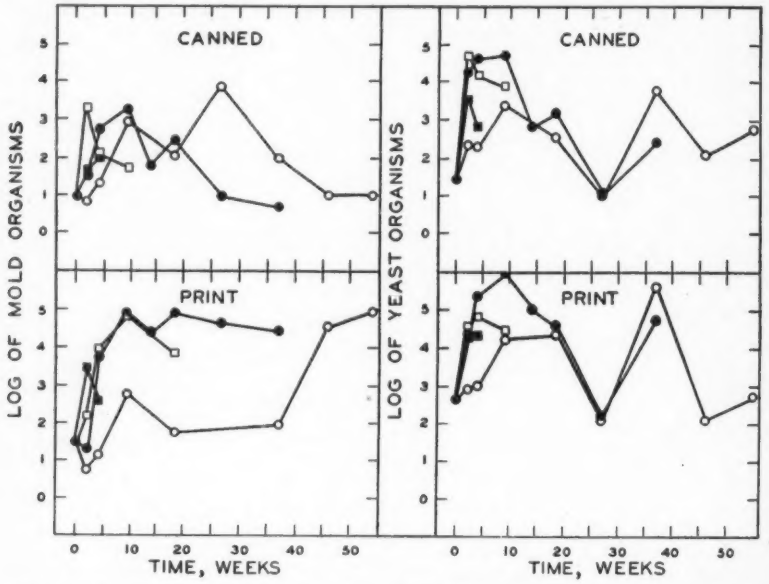

FIG. 4. *Effects of storage at 30°, 50°, 70°, and 90°, F. on mold and yeast organism contents of canned and print, salted butter. 30° F., ○; 50° F., ●; 70° F., □; 90° F., ■.*

reaching a maximum in 16 to 32 days, and then decreasing after approximately 64 days. At 30° F., the trends were more uniform and the peaks were reached in from 32 to 70 days and in some cases even longer. In general, the changes in the canned butter were not so great as in the print and the organisms did not survive so long. In the prints, the number of organisms usually remained at a level as great as or greater than the original number, whereas in the canned butter the levels were usually below the original values.

The numbers of total viable organisms (Fig. 3) at both 70° and 90° F. rose rapidly, the increase in the prints being about 10 times greater than in the canned. In the butter stored at 50° F., the increase was more rapid in the prints and greater numbers were attained. At 30° F., there was little increase in the canned material, but in the prints the increase was decided and the number of organisms remained at a high level throughout the experiment.

The number of proteolytic organisms showed trends similar to those of the viable count (Fig. 3). At 30° F., there was very little increase. The decreases after the initial increases were somewhat steeper than for the total count.

The oxidase producing organisms increased at a much slower rate in the canned butter than in the prints (Fig. 3). However, in the canned material the numbers eventually decreased at the higher temperatures to levels equal to or lower than the original number. For the prints the decrease was slower, but counts finally reached the original level, except in the prints stored at 30° F.

Lipolytic organisms increased from a low original content of about 140 per gm. to about a million per gram in the print butter stored at 50° and 70° F. (Fig. 3). At the three lower storage temperatures, the average number remained at above 10,000 per gram for 20 weeks. In the cans the increase, although not great, was maintained over a similar period, and the number then dropped to approximately the original value.

The yeast count showed a greater increase in the canned butter than in the prints (Fig. 4). For both the print and canned butter stored at 30° F., there was a general increase for at least the first 35 weeks. The drop shown at the 27th week is due to a poor lot of media. Development of molds was much greater in the prints than in the canned material. This is readily seen from Fig. 4. The counts remained high in the prints, but in the canned butter there was some reduction after the first few weeks. Darkening of the surface due to molds was first noticed in the prints in 32 days at 70° F., in 64 days at 50° F., and in 192 days at 30° F. Light mold growth was noted a few times on the surface of the canned butter but never became distributed throughout the mass as in the prints.

Relation Between Flavor and Objective Test Values

The relation between flavor as assessed by the laboratory flavor panel and objective test values for each storage temperature was assessed by computing correlation and regression coefficients. An objective test, to be of value for general assessment of keeping quality, should have a correlation coefficient of at least .8 and there should be no significant difference in regression values between sets of data from material stored under different conditions. The significance of any difference in regression values for the butter stored at the four temperatures was determined by analysis of covariance.

The correlation coefficients of the relations between flavor and the objective tests are given in Table IV. All the objective test values increased with a decrease in the flavor score, except pH, which decreased. The chemical objective tests were more closely related to flavor changes than the bacteriological tests. The correlation coefficients between flavor and viable, proteolytic, oxidase, and yeast counts attained significance for storage at 30° and 90° F. while those for 50° and 70° F. were not significant. Lipolytic count correlated significantly with flavor only for 90° F. storage. Of all the bacteriological tests studied, the mold count gave the highest correlation coefficient with flavor and attained statistical significance for all temperatures except 30° F. Although the correlation coefficient between flavor and mold count was much higher than for the other bacteriological tests, they were still too

TABLE IV

SIMPLE COEFFICIENTS OF CORRELATION BETWEEN FLAVOR SCORES AND OBJECTIVE TEST VALUES FOR SALTED BUTTER STORED AT 30°, 50°, 70°, AND 90° F.

Quantities correlated:	30° F.		50° F.		70° F.		90° F.	
Flavor score with:	Degrees of freedom	r	Degrees of freedom	r	Degrees of freedom	r	Degrees of freedom	r
Viable count	29	−.56**	35	−.14	39	−.07	39	−.33*
Proteolytic count	28	−.36*	35	−.01	39	−.07	39	−.44**
Lipolytic count	29	−.27	35	−.10	38	−.12	39	−.46**
Oxidase positive count	29	−.45**	35	.01	39	−.23	39	−.34*
Yeast	29	−.59**	35	−.24	37	−.01	39	−.37*
Mold	29	−.30	35	−.84**	38	−.65**	39	−.36*
Butter acidity	30	−.59**	38	−.71**	41	−.79**	36	−.67**
Free fatty acids	30	−.61**	38	−.82**	41	−.71**	36	−.21
pH	30	.63**	38	.84**	41	.82**	36	.51**
Fluorescence	30	−.63*	38	−.69**	40	−.75**	36	−.84**
Amino acid	20	−.28	26	−.50**	22	−.61**	20	−.75**
Free serum acidity	20	−.75**	26	−.92**	22	−.67**	20	−.71**
Total serum acidity	20	−.82**	24	−.72**	26	−.86**	20	−.64**

* *Indicates 5% level of statistical significance.*
** *Indicates 1% level of statistical significance.*

low to predict flavor score with any degree of reliability. The whole butter acidity, free fatty acids, and pH showed higher correlation with flavor for the material stored at 50° and 70° F. than for that stored at 30° and 90° F. Correlation coefficients between flavor and serum fluorescence and amino acids increased with an increase in storage temperature. Of the chemical tests studied, free serum acidity and total serum acidity gave the highest correlation coefficients with flavor.

The regression coefficients between flavor and serum fluorescence, whole butter acidity, free fatty acids, free serum acidity, amino nitrogen, total serum

TABLE V

REGRESSION COEFFICIENTS BETWEEN FLAVOR SCORES AND OBJECTIVE TEST VALUES FOR SALTED BUTTER STORED AT 90°, 70°, 50°, AND 30° F.

Quantities correlated:	90° F.		70° F.		50° F.		30° F.	
Flavor score with:	Degrees of freedom	β	Degrees of freedom	β	Degrees of freedom	β	Degrees of freedom	β
Free fatty acids (whole butter)	36	− .087	41	−.027	38	−.017	30	−.058
Free fatty acids (butter fat)	36	− .019	41	−.021	38	−.016	30	−.054
pH on serum	36	1.35	41	2.94	38	3.49	30	2.70
Fluorescence value on serum	36	− .064	40	−.160	38	−.207	30	−.100
Amino nitrogen on serum	20	− .066	22	−.104	26	−.074	20	−.046
Free serum acidity value	20	− .060	22	−.073	26	−.061	20	−.132
Total serum acidity value	20	− .040	22	−.052	26	−.043	20	−.069

acidity, and pH on the serum for each storage temperature are given in Table V. It is quite evident that a difference in regression coefficients exists between the storage temperatures and the regression coefficients for pH are greater than for the other measurements. The significance of these differences was assessed by analysis of covariance (Table VI). No significant

TABLE VI

ANALYSIS OF COVARIANCE WITH STORAGE TEMPERATURES OF REGRESSION COEFFICIENTS BETWEEN OBJECTIVE TESTS AND FLAVOR SCORE

Tests with flavor	Average regression coefficient		Individual coefficient		Difference in regression coefficients		Residual (pooled)	
	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Fluorescence	1	235.42**	4	72.99**	3	18.85**	146	1.96
Free fatty acid (whole butter)	1	228.50**	4	67.23**	3	13.48**	138	1.90
Free fatty acid (butterfat)	1	242.36**	4	69.99**	3	8.53**	138	1.89
Free serum acidity	1	265.95**	4	69.78**	3	4.39	94	2.09
Amino nitrogen	1	127.19**	4	33.81**	3	2.68	94	3.65
Total serum acidity	1	263.60**	4	67.53**	3	2.16	94	2.18
pH on serum	1	334.39**	4	119.42**	3	47.77**	154	0.73

** *Indicates 1% level of statistical significance.*

difference in regression coefficients between storage temperatures was found for the relations between flavor score and free serum acidity, amino nitrogen, and total serum acidity. Significant differences between the storage temperatures were evident for the regression coefficients between flavor and whole butter acidity, free fatty acids, pH, and serum fluorescence.

Interrelation of the Chemical Measurements

The interrelation of the chemical measurements was assessed by computing correlation coefficients. The results are shown in Table VII. The fluorescence values were most highly associated over all the storage temperatures with amino acids and total serum acidity, and least with pH. From these results, it may be inferred that fluorescent materials are derived from amino acids or a reaction in which amino acids are involved. Free serum acidity and amino acids were not so closely associated as the amino acids and total serum acidity. This indicates that free serum acidity may be produced by microbiological action without significant production of amino acids. The pH was significantly correlated with all measurements but least so with amino acid content. A storage temperature of 30° F. gave a much lower association between the free fatty acids and other measurements except whole butter acidity. The acids content of the whole butter and butterfat were significantly correlated with the other measurements.

TABLE VII

SIMPLE CORRELATION COEFFICIENTS FOR THE INTERRELATION BETWEEN OBJECTIVE TESTS ON BUTTER STORED AT 30°, 50°, 70°, AND 90° F.

	Storage temp., °F.	Correlation coefficients					
		Butter acidity	Free fatty acids	pH	Amino acids	Free serum acidity	Total serum acidity
Fluorescence	90	.65**	.76**	−.21	.85**	.79**	.92**
	70	.82**	.90**	−.48**	.86**	.71**	.83**
	50	.70**	.77**	−.50**	.84**	.64**	.77**
	30	.70**	.72**	−.26	.67**	.59**	.79**
Total serum acidity	90	.87**	.93**	−.85**	.89**	.89**	
	70	.77**	.78**	−.76**	.88**	.95**	
	50	.82**	.90**	−.77**	.80**	.83**	
	30	.42*	.61**	−.27	.80	.77**	
Free serum acidity	90	.87**	.77**	−.84**	.58**		
	70	.75**	.81**	−.80**	.69**		
	50	.40	.91**	−.17	.34		
	30	.53**	.74**	−.74**	.25		
Amino acids	90	.66**	.62**	−.60**			
	70	.55**	.57**	−.54**			
	50	.58**	.63**	−.34			
	30	.23	.20	−.28			
pH	90	−.87**	−.60**				
	70	−.62**	−.92**				
	50	−.70**	−.73**				
	30	−.53**	−.55**				
Free fatty acid	90	.78**					
	70	.96**					
	50	.86**					
	30	.90**					

* *Indicates 5% level of statistical significance.*
** *Indicates 1% level of statistical significance.*

Discussion

Many investigators have employed mold, yeast, and bacterial counts to assess the keeping quality of butter. In the present investigation, temperature had a pronounced effect on the association between microorganisms and flavor. Higher correlations were found between flavor score and the numbers of proteolytic, lipolytic, and oxidase producing organisms at 30° and 90° F. than at 50° and 70° F. This finding, and the fact that considerable difficulty is encountered in estimating the true number of organisms present, make it unlikely that any microbiological test employing present methods would prove to be satisfactory for predicting flavor score.

The measurements on the serum fraction were the most promising of the chemical methods investigated for predicting flavor score during storage. In a previous investigation (10) fluorescence measurements of the serum showed possibilities as a test for estimating keeping quality. The present evidence

confirms its usefulness as a guide for investigational purposes, especially under accelerated storage conditions (70° and 90° F.). However, the flavor tests remain the best method of estimating keeping quality.

The free serum acidity, amino acids, and total serum acidity showed no significant differences in regression coefficients between storage temperature, indicating that the rate of formation of acids with flavor score decrease was the same for all the storage temperatures studied. This is one of the essential requirements of an objective test of flavor deterioration. However, the amino acids showed low correlation coefficients at some of the storage temperatures, which prevents their use as a keeping quality test. The remaining two tests were closely associated with flavor deterioration, but no prediction equations were calculated, as it was deemed desirable to base such equations on data from samples obtained from many sources and stored under varying temperatures and humidities. Moreover, because of the complexity of the reactions involved in butter deterioration, it would seem desirable to use more than one test in constructing prediction equations for assessing keeping quality.

Of the objective tests used on the serum fraction the commonly used pH measurement was useful for assessing flavor deterioration in butter stored at low temperatures but not in butter stored at high temperatures. Free serum acidity, total serum acidity, and fluorescence may be applied under accelerated storage conditions to differentiate between butters of good and poor keeping qualities.

As no appreciable oxidative decomposition of the butterfat could be detected by tests of peroxide oxygen or aldehyde content, the free fatty acids of the butterfat were evidently developed by the action of lipolytic enzymes. This lack of appreciable increase in peroxide oxygen and aldehyde content indicates that oxidative changes in the fat fraction contribute little to flavor deterioration in stored salted butter. However, the free fatty acids developed by lipolysis may exert some influence at high storage temperatures.

Decomposition of the serum and flavor deterioration were evident before any free fatty acid or oxidative changes could be detected in the fat. As shown by other workers (4), the antioxidant effects of decomposition products of the serum, e.g., amino acids, may account for the stability of the fat fraction. Flavor deterioration is evidently due to protein decomposition rather than to changes in the fat fraction. The decrease in association between flavor score and amino acids with a decrease in storage temperature indicates that the serum protein was not so completely decomposed at the lower temperatures, that intermediate products are responsible for flavor deterioration, and that deterioration reactions are not the same at all temperatures.

From the results of the chemical and microbiological tests it is apparent that deterioration is retarded in canned butter, especially at the lower temperatures studied. Indeed, canned butter can be stored at 30° F. for periods up to 45 weeks without appreciable lowering of its quality. Therefore canned butter can be stored and transported at chill temperatures rather than at the freezing temperatures required for butter in prints.

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APPENDIX TABLE I

FLAVOR PANEL SCORES AND OFFICIAL GRADERS' SCORES OF BUTTER FROM PLANT A

Storage temperature, °F.	Storage time, days	Canned		Print	
		Panel	Graders	Panel	Graders
30	Initial	7.2	39.0	7.5	38.5
	16	7.7	40.0	7.2	39.5
	32	7.5	38.5	5.9	38.0
	64	6.1	38.0	3.7	39.0
	128	7.4	39.0+	4.3	39.5
	192	6.6	39.0	4.1	—
	256	7.6	38.0	4.0	36.0−
	321	6.5	39.0	2.8	37.0
	385	6.9	—	0.0	—
50	Initial	7.2	39.0+	7.5	38.5
	4	7.3	39.0	7.4	39.0
	8	7.0	39.0	6.9	38.5
	16	7.1	39.0−	6.8	39.5
	32	6.5	36.5	5.6	38.5
	64	3.3	37.0	0.9	36.0
	96	4.9	36.0	2.4	36.0
	128	4.9	—	0.7	—
	192	6.1	37.5	2.0	—
	256	5.9	38.0	0.0	—
70	Initial	7.2	39.0+	7.5	38.5
	0.5	7.3	38.5	7.4	38.5+
	1	7.0	39.0−	6.9	39.0
	2	7.3	39.0−	7.2	38.5
	3	7.0	39.0	6.7	39.0−
	4	6.8	39.0	6.9	39.0−
	8	6.5	37.5	6.2	39.0+
	16	6.1	38.5	6.1	37.5
	32	5.1	37.0	4.4	36.5
	64	3.2	38.0	1.2	35.0
	128	4.2	—	1.6	—
90	Initial	7.2	39.0+	7.5	38.5
	0.25	7.4	39.0+	7.5	39.5
	0.5	—	37.0	—	38.5
	1	6.7	38.5	6.5	39.0
	2	6.0	39.0−	6.2	38.5
	3	6.3	39.0	6.4	39.0−
	4	6.3	39.0−	6.2	38.5
	8	5.3	38.0−	5.2	38.0+
	16	4.6	37.5+	5.0	38.5−
	32	3.6	39.0−	4.7	39.0

APPENDIX TABLE II

FLAVOR PANEL SCORES AND OFFICIAL GRADERS' SCORES OF BUTTER FROM PLANT B

Storage temperature, °F.	Storage time, days	Canned		Print	
		Panel	Graders	Panel	Graders
	Initial	8.9	39.0−	8.7	39.0
	16	7.4	39.5+	6.9	39.0−
	32	7.2	39.5	6.9	39.0+
	64	7.5	39.0	6.4	36.0
30	128	7.6	39.0−	4.2	38.0
	192	7.4	37.0	2.6	—
	256	6.3	37.0	3.8	—
	321	6.9	39.0−	3.4	36.0
	385	4.8	—	0.0	—
	Initial	8.9	39.0−	8.7	39.0
	4	6.9	39.0	7.2	39.0
	8	7.1	39.0	7.4	38.5
	16	7.4	39.5−	5.9	39.0+
	32	5.1	38.0	4.5	37.0
50	64	6.6	39.0	2.3	36.5
	96	4.6	38.0	1.5	36.0
	128	6.3	39.0	1.5	36.0
	192	6.5	38.0	1.2	—
	256	5.5	37.0	0.0	—
	Initial	8.9	39.0−	8.7	39.0
	0.5	7.8	38.5	7.8	39.0
	1	7.7	39.5	7.7	39.0
	2	7.1	39.5	7.7	39.0
70	3	7.5	39.5−	6.4	39.0
	4	6.9	39.0	6.9	39.0−
	8	6.1	37.5	7.2	37.5
	16	6.7	39.0	5.1	38.0−
	32	4.4	35.0	2.8	36.0
	64	2.5	35.5	1.0	36.0
	128	3.3	—	2.6	—
	Initial	8.9	39.0−	8.7	39.0
	0.25	7.3	38.5	7.2	39.0−
	0.5	7.0	38.0	7.2	38.0
	1	6.5	38.5	6.9	38.0
	2	6.5	38.0	6.1	38.0
90	3	6.0	38.0+	4.8	38.0−
	4	6.0	39.0−	5.2	38.0
	8	6.5	37.5	4.1	37.0
	16	5.3	38.5	3.8	37.0−
	32	5.2	39.5+	4.2	39.5−

THE OXIDATION, IGNITION AND DETONATION OF FUEL VAPORS AND GASES

III. THE CAUSE OF THE EFFECT OF METALLIC DOPE TO DELAY DETONATION[1]

By R. O. King[2]

Abstract

The antiknock effect of metallic dope is attributed to the catalytic action of the surface, maintained in an oxidizing atmosphere by continuous deposition thereon of the metal of the dope, to oxidize the fuel in part to steam and carbon dioxide, the consequent dilution of the end gas causing a reduction of inflammability sufficient to prevent completion of combustion in other than the normal manner. Experiments indicate that temperatures required for the necessary catalytic activity are attained during the period of flame travel; not prior to ignition. In support of the theory experimental evidence is given showing that, (*a*) steam is especially effective to reduce inflammability, (*b*) doped pentane or hexane oxidizes without ignition to steam and carbon dioxide only in reaction chamber No. 10, described in Part I, at any temperature of reaction, (*c*) the carbon dioxide–steam reaction is inhibited by a sulphur catalyst poison and the similar action in the engine destroys the antiknock effect of the dope. It is shown further by experiments with and without additions of a catalyst poison to *undoped* engine fuel that, in conditions leading to high surface temperatures in the combustion space, a carbon dioxide–steam reaction having a considerable antiknock effect is obtained. The engine experiments with catalyst poisons are completed by obtaining conditions in which tetraethyl lead becomes a pro-knock. It is recognized that the catalyst theory depends fundamentally on the oxidation of hydrocarbon fuel being a heterogeneous reaction as indicated by the experiments described in Part II, and some further evidence in support of the view, obtained by using catalyst poisons, is given in this Part.

Introduction

The discovery by Midgley and Boyd of the remarkable effect of tetraethyl lead present in extremely small concentration in engine fuel, to delay the onset of detonation as the compression ratio is raised, was followed by a spate of explanatory theories, the number extending ultimately to more than a dozen. Those proposed prior to 1926 and reviewed by Clark and Thee (5) did not involve a departure from accepted belief concerning the mechanism of the oxidation of the paraffin hydrocarbons present in large proportion in normal engine fuel and mainly responsible for the occurrence of detonation.

Such a departure was made in the Callendar nuclear drop–peroxide theory (3, 4) submitted to the Aeronautical Research Committee, December, 1926, in that oxidation was taken to begin with the formation of an organic peroxide instead of an hydroxyl compound as required by the generally accepted, Armstrong–Bone hydroxalation theory. Support was afforded the peroxide theory by the work of Moureu, Dufraisse, and Chaux who followed Callendar in associating peroxides with fuel detonation (15).

[1] *Manuscript received in original form August 11, 1947, and, as revised, November 25, 1947. Contribution by Defence Research Board (Canada).*

[2] *Research Scientist, Naval Division; lately Director of Scientific Research and Development, Royal Canadian Navy.*

Egerton and Gates (6), in a review of the theories of antiknock action, agreed that peroxides, acting as autocatalysts, are formed in the early stage of combustion and suggested that metallic antiknocks form comparatively stable peroxides which react with and destroy the fuel peroxides which are then regenerated. The suggestion led Egerton (7, pp. 2911–2926) to the formulation of a chain reaction theory in which peroxide is an essential link, the development of the reaction to cause knocking being interrupted on the destruction of the peroxide by the metal of the dope, which is oxidized in the process. Finally, Beatty and Edgar (2, pp. 2927–2936) in a comprehensive review of the theory of knock in internal combustion engines conclude that "antiknock agents act as inhibitors for the slow oxidation reactions in the end gas. Their effect is to break short the reaction chains and so prevent the chain development from reaching that critical point at which its multiplication begins".

Theories that antiknocks act by destroying organic peroxides or by breaking reaction chains in an homogeneous fuel–air mixture are not accepted but discussion can be reserved. The reviews mentioned do, however, deal with two matters related to experimental work described in Parts I (8) and II (9) and later in this Part.

Beatty and Edgar mention particularly the difficulty of relating oxidation as it occurs in laboratory experiments to the similar effect in the end gas of an engine. Thus it is stated that laboratory oxidations cannot be carried to temperatures higher than 500° C. because of the incidence of ignition or explosion, and that time of reaction is sometimes 1000-fold greater than in an engine. The statements were, of course, made before the development of reaction chamber No. 10, described in Part I (8) in which it is possible to carry laboratory oxidations of hydrocarbons commonly used as engine fuel to whatever temperature is required for completion, 700° C. or over, without ignition and in reaction times of a second or less.

The positive catalyst theory, which assumes that antiknocks accelerate oxidation *prior to ignition* in the engine and accordingly weaken the mixture to an extent sufficient to prevent detonation, is mentioned by Egerton with the comment that the antiknocks retard oxidation and consequently the theory is contrary to fact. Beatty and Edgar also mention the theory and dismiss it on sounder grounds by observing, in effect, that promotion of the oxidation of the fuel by antiknocks during the *early stages of combustion* in an engine is not in accordance with experimental facts.

Revised Positive Catalyst Theory

The experiments described in Part I (8) and others to be described in this Part show that the oxidation of paraffin hydrocarbons doped with iron carbonyl, in reaction chamber No. 10, which provides conditions approaching those obtaining in the combustion space of an engine, is a heterogeneous reaction on a surface of iron maintained as such in an oxidizing atmosphere by continuous deposition of metal from the organic compound. The final

products only, namely steam and carbon dioxide, are produced at any temperature of reaction. The reaction does not proceed with high velocity until surface temperature exceeds 500° C. Such high surface temperatures would be found in the combustion space of an engine on the exhaust valves in conditions of maximum power output and momentarily on other surface exposed to radiation from the advancing flame which reaches temperatures of 2500° C. and over, depending on compression ratio and other factors. The end gas in which detonation occurs is heated by compression to temperatures of 650° C. and over, depending on compression ratio, and would be expected to attain even higher temperatures because of turbulence. Temperature and turbulence conditions are, therefore, as required to promote extremely rapid surface oxidation of the doped fuel to final products which could reduce the inflammability of the end gas to such an extent that completion of combustion would be possible solely by normal flame travel. An adequate explanation for the antiknock action of metallic dopes is then obtained without having to determine the cause of detonation.

Section I

Effect of Steam to Reduce Inflammability

The effect of steam, nitrogen, and carbon dioxide as diluents on the inflammability of a normal fuel–air mixture used in the E 35 engine was determined by Ricardo (17). The work was extended and the experimental results discussed some time later by Alcock (1). The gases mentioned were added to the fuel–air charge in increasing proportion until inflammability was so reduced that misfiring occurred. It was emphasized that elaborate precautions, including superheating, were required to ensure that steam was added to the engine charge as such, not as part water.

It was found that 8.9% of steam sufficed to reduce inflammability to the extent required to cause misfiring although flame temperature was reduced by 125° C. only. Carbon dioxide was required in the proportion of 19.6% to produce a similar effect although flame temperature was reduced by 400° C. The conclusion was that steam exerted a nonthermal effect to reduce inflammability, the nature being unknown. Whatever the nature of the effect may be, the magnitude is of great importance, and from the data given above it is calculated that, on a weight basis, steam is six times more effective than carbon dioxide, the other product of the complete combustion of hydrocarbons.

Effect of Steam to Reduce the Inflammability of Hydrogen

Hydrogen is especially suitable for experiments to determine the effect on inflammability of steam which is the sole final product of oxidation. It was mentioned, Part I (8), that tetraethyl lead and iron carbonyl had been shown by Mardles to promote the oxidation of hydrogen. The rate of oxidation was not, however, related to inflammability, and the experiment using the carbonyl as the oxidant has been repeated in carefully controlled

conditions and using a conventional flow method. The combustion tube was of silica, 1 in. internal diameter, supported in a vertical 6 in. furnace. Silica was selected because of its relatively inactive surface. The hydrogen and air were from high pressure cylinders and, therefore, nearly dry, but, as a further precaution, the gases were passed over phosphorus pentoxide while en route to the combustion tube. The reacting mixture was supplied at the rate of 200 cc. per min. and contained hydrogen in the concentration of 40% by volume. Iron carbonyl, as vapor, was added to the air stream at the rate of 40 mgm. per hour. Temperatures were taken by means of a thermocouple in a silica sheath placed at the position of maximum temperature on the longitudinal axis of the combustion tube. The hot gases from the combustion tube were passed through a Liebig condenser and then through a U-tube

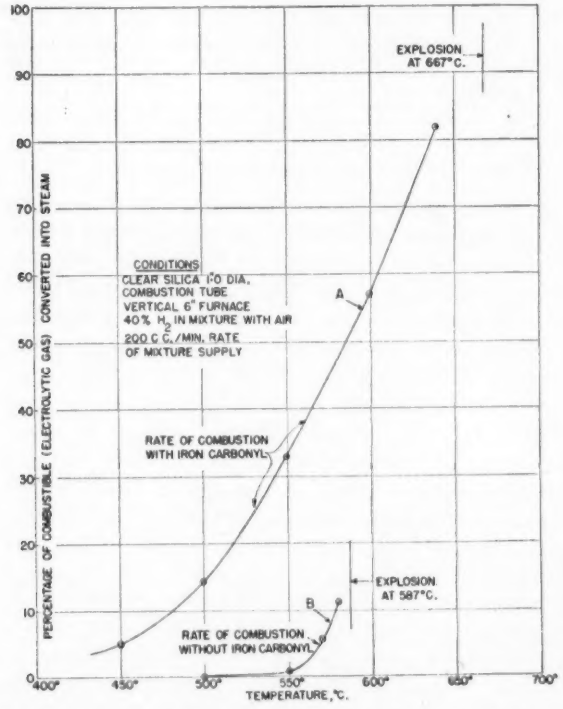

FIG. 1. *Graphs showing the effect of steam from a heterogeneous reaction to reduce inflammability and thereby raise explosion temperature.*

containing calcium chloride. Rates of oxidation were measured by weighing the water of combustion at suitable time intervals. When iron carbonyl was added to the reacting mixture, iron oxide was deposited on the tube at the positions of the upper and lower convection circulation, see Fig. 5, Part I (8), and in view of the fact that the mixture contained both oxidizing and reducing

gases, care was taken that the equilibrium oxide had formed prior to measuring rate of combustion. Equilibrium was taken as having been established at a particular temperature when water of combustion was obtained at a constant rate.

Graph *A* of Fig. 1 gives rates of steam formation when the hydrogen–air mixture contained the vapor of iron carbonyl, and Graph *B*, rates of steam formation when the mixture without the vapor was passed through the tube in clean condition. It will be noted that with iron carbonyl present combustion of electrolytic gas occurred at the easily measurable rate of 5%, at 450° C., whereas the rate was about 1% only at 550° C., in the absence of the carbonyl and 33-fold greater when it was present. The mixture containing iron carbonyl exploded at 667° C. and at 587° C., in the absence of the carbonyl.

Concluding Comments

The experiments by Ricardo and Alcock show the effect of steam from an outside source to reduce the inflammability of the reacting mixture in an engine. Those just described show a similar effect obtained by steam produced by the reaction itself.

Section II

The Oxidation of Pentane in Reaction Chamber No. 10, as Affected by Iron Carbonyl

The reaction chamber was described in Part I (8), and experiments on the effect of flow configuration described in Part II (9) indicated that oxidation in the chamber is a heterogeneous reaction so far as doped pentane is concerned and probably of a similar character in respect of pentane alone. Experiments described in this section were made to confirm the earlier results and to determine the effect on rate of oxidation of changing the concentration of iron carbonyl and the effect of a residual surface of iron oxide.

The experimental results shown by the graphs of Figs. 7, 8, 9 and 10 of Part I (8) were obtained immediately after the completion of the reaction chamber. The chamber was then dismantled, cleaned with hydrofluoric acid, washed with steam, and an oxidation of pentane containing iron carbonyl in the concentration of 0.5% repeated. The experimental results are plotted on Graph *B* of Fig. 2, together with those obtained earlier, and it will be noted that the two sets of results fall on a single curve showing a degree of repeatability not generally obtained when using conventional methods.

The increase in rate of reaction obtained on doubling the dope concentration is shown by Graphs *B* and *C* of Fig. 2. A useful comparison can be made at the temperature of 550° C., when about half of the oxygen remained uncombined. The increase was from 4.1 to 5.5 moles of oxygen reacted, that is, 30%. Flow configuration was similar for both experiments and the increase is attributed to some possible increase in the area of the effective catalytic iron surface or to an increase in the density of the catalyst.

Rates of formation of carbon oxides for the smaller concentration of iron carbonyl were given by the graphs of Fig. 8, Part I (8). Exactly similar results were obtained when using the larger concentration and as before reaction was substantially to steam and carbon dioxide whatever the temperature.

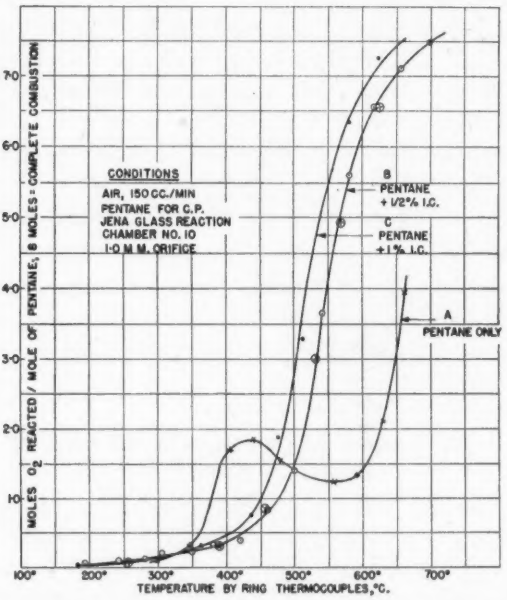

FIG. 2. *Graphs showing the increase in velocity of the carbon dioxide–steam reaction due to doubling the iron carbonyl concentration in pentane.*

Pentane Oxidation on Residual Oxide Surface

The coating of the surface of the reaction chamber with iron and iron oxides seen after oxidation of pentane doped with iron carbonyl was shown, *B*, Fig. 6 of Part I (8); distribution varies with rate of mixture supply and the concentration of iron carbonyl in the pentane and thickness with time of use of the doped pentane, but the surface becomes completely coated with brick red oxide on continued use of the chamber with undoped mixture. The results of an oxidation of pentane after use of the chamber with pentane containing iron carbonyl in the concentration of 1.0% are given by Graph *C* of Fig. 3. Graph *A* for pentane oxidized in the chamber when clean and Graph *B* for the oxidation of pentane plus 1.0% iron carbonyl are given for ease of comparison. The graphs show that when the metal surface was no longer maintained by continuous deposition from the carbonyl, rates of reaction at temperatures above 500° C. are greatly reduced and tend to approach those obtained when the reaction chamber surface is clean glass. Thus at 550° C. rate of reaction fell from 5.2 moles, Graph *B*, to 1.8 moles of oxygen reacted, Graph *C*. At 625° C., oxygen was reacted at the rate of

7.25 moles when using the doped mixture, Graph *B*, but diminished to 5.8 moles for the residual surface, Graph *C*, and further to 4.6 moles after 3½ hr., as shown by the single observation marked with an arrow below Graph *C* of the figure.

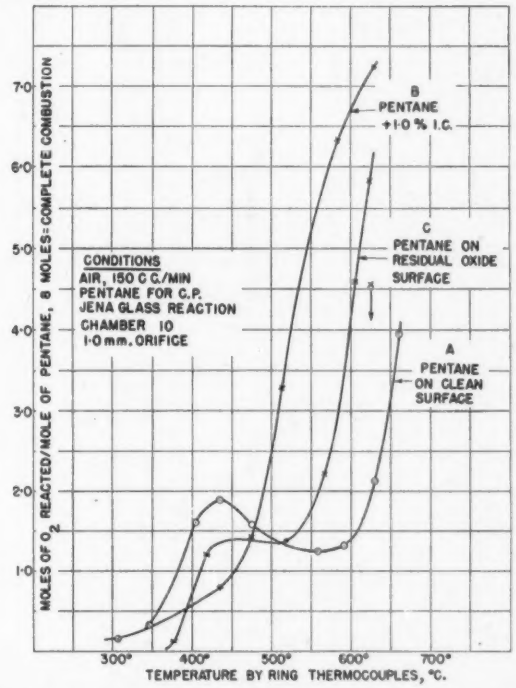

FIG. 3. *Graphs showing the effects on the oxidation of pentane of ceasing to maintain the catalytic surface provided by continuous deposition of iron from the carbonyl.*

The reappearance of the aldehyde hump, Graph *C* of Fig. 3 when the active metal surface of the reaction chamber surface was converted to oxide is of great interest and supports the view, expressed in Section V of Part II (9), that the oxidation of pentane to partial products, including aldehyde, is a heterogeneous reaction.

Section III

THE OXIDATION OF HEXANE IN REACTION CHAMBER NO. 10, AS AFFECTED BY IRON CARBONYL

Hexane is present in considerable proportion in normal engine fuel and is of relatively low octane number. Experimental results for the effect of metallic antiknocks on the oxidation of the substance, hitherto published, have always been obtained by conventional methods and because of the almost complete oxidizing nature of combustible mixtures with air, reaction could not occur on a surface of metal deposited from the dope, except at very

low temperatures. The consequent effect of the dope to inhibit the oxidation of hexane and higher paraffins in the same circumstances is the experimental basis of current theory attributing the antiknock action to an antioxidant property. It became of special interest, therefore, to determine if the oxidation of hexane in reaction chamber No. 10 at high temperatures would be promoted on the addition of metallic dope and if reaction would be to final products as for pentane oxidation.

Oxidations in Reaction Chamber No. 10

The experimental conditions were as described for oxidations of pentane. The 'hexane', supplied by the Anglo-American Oil Company, was procured from the fractional distillation of nearly aromatic-free petroleum spirit. The density was 0.6965, and distillation range 62° to 85° C. for a 95% fraction.

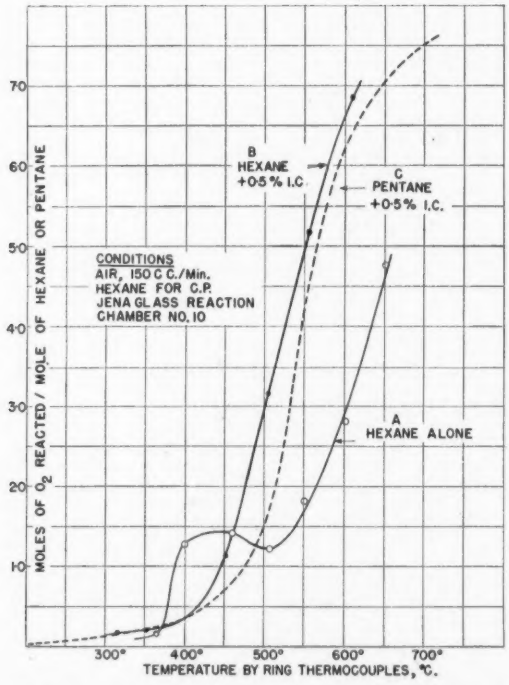

FIG. 4. *Graphs showing the effect of iron carbonyl to promote the oxidation of hexane at high temperatures as compared with the similar effect for pentane.*

Experimental results are given by the graphs of Fig. 4: Graph *A* is for hexane alone in the reaction chamber in clean condition; Graph *B* is for hexane plus iron carbonyl in the concentration of 0.5%. The combining proportions mixtures used for the experiments were made up on the assumption that the hexane was normal and that 9½ moles of oxygen would be required accordingly

for the complete oxidation of one mole of the paraffin. It will be noted that the characteristics of hexane oxidation are similar to those determined for the oxidation of pentane. Graph *C* of the figure, for the oxidation of doped pentane, is reproduced from Fig. 2 and appears to show that iron carbonyl possesses the greater oxidation promoting effect in respect of pentane, but, when allowance is made for the specific oxygen consumption, the carbonyl is found to be of equal effect in respect of the two paraffins, as nearly as can be determined without exact knowledge of molecular composition.

The oxidation of doped hexane was, as for doped pentane, substantially to steam and carbon dioxide at any temperature of reaction, as shown by the graphs of Fig. 5.

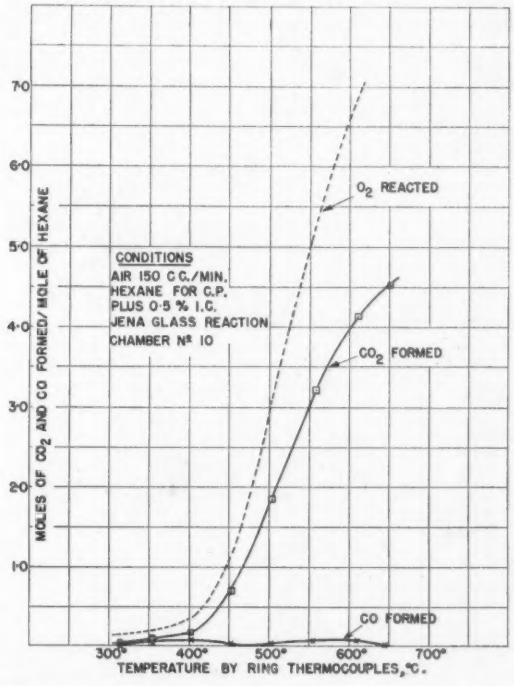

FIG. 5. *Graphs showing that the oxidation of hexane containing iron carbonyl is substantially to carbon dioxide and steam at all temperatures of reaction.*

Section IV

THE SPECIFIC EFFECT OF ETHYL MERCAPTAN TO INHIBIT THE HETEROGENEOUS REACTION OF PENTANE AND HEXANE TO STEAM AND CARBON DIOXIDE

Having shown that the antiknock, iron carbonyl, suppresses the formation of partial oxidation products, including aldehyde, and promotes the formation of steam and carbon dioxide at all temperatures of reaction, it remains to

determine which of the two effects is responsible for the antiknock property of the carbonyl. The use of a catalyst poison in oxidation and related engine experiments makes it possible to distinguish between the effects mentioned.

Ethyl Mercaptan and Hydrogen Sulphide

The one hydrogen atom of the mercaptan and the two of the sulphide are replaceable by metal. Either substance converts a contact surface of iron to one of iron sulphide and is without effect on a surface of iron oxide. Both substances were known from earlier experiments to destroy the antiknock effect of the metallic dopes. The mercaptan, rather than hydrogen sulphide, was used for laboratory experiments to avoid the complications involved in the supply and measurement of the sulphide at the small rates required. The concentration of 2.5% by weight of the mercaptan in the pentane and hexane is without significance except that in engine experiments it was sufficient to destroy the antiknock effect of 2 cc. per gal.* of tetraethyl lead in an engine fuel.

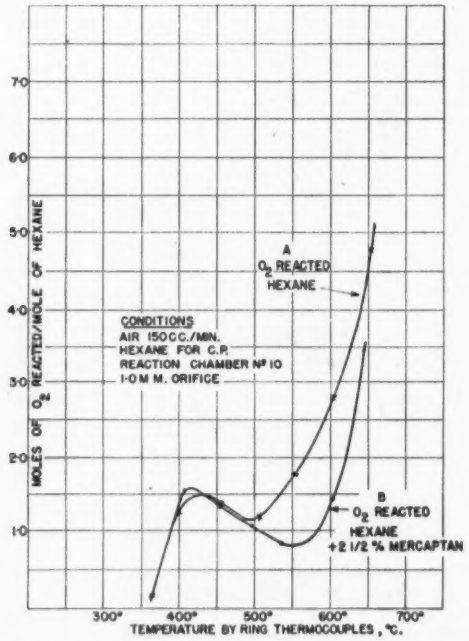

FIG. 6. *Graphs showing that ethyl mercaptan, a catalyst poison, does not affect the aldehyde reaction in a glass reaction chamber (No. 10).*

Effect of Ethyl Mercaptan on the Oxidation of Hexane in Reaction Chamber No. 10

The experimental results are given by the graphs of Figs. 6, 7, and 8. It is of special significance, as shown by the graphs of Fig. 6 for oxygen reacted,

* *References throughout this paper are to the imperial gallon.*

that the mercaptan had no measurable effect on reaction velocities at temperatures below about 480° C., that is, at the temperatures of the formation of partial products including aldehyde. The inhibitory effect was confined to the carbon dioxide–steam reaction as shown by the graphs of Figs. 7 and 8 for rates of formation of carbon oxides.

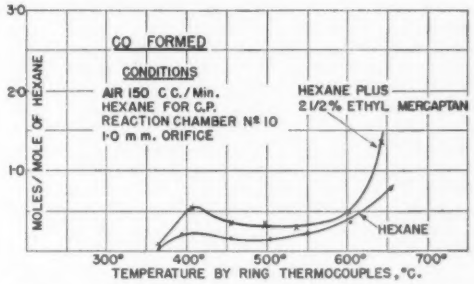

FIG. 7. *Graphs showing the effect of ethyl mercaptan on carbon monoxide formation when hexane is oxidized in reaction chamber No. 10.*

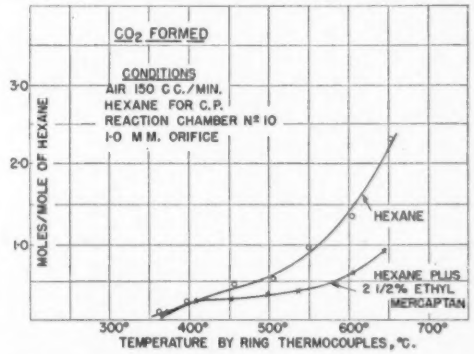

FIG. 8. *Graphs showing the effect of ethyl mercaptan on carbon dioxide formation when hexane is oxidized in reaction chamber No. 10.*

Effect of Ethyl Mercaptan on the Oxidation of Hexane plus Iron Carbonyl in Reaction Chamber No. 10

The experimental results are given by the graphs of Figs. 9, 10, and 11. At 550° C. oxygen was reacted at the rate of 5.0 moles when the pentane contained 0.5% of iron carbonyl, Graph *A*, Fig. 9. The rate was 1.2 moles only when the doped pentane contained $2\frac{1}{2}$% of mercaptan. Carbon dioxide, at the same temperature, was formed at the rate of 1.75 moles without the mercaptan and 0.25 moles with it. Carbon monoxide formation, Fig. 11, was inappreciable in either case. At 600° C. oxygen reacted was 6.6 moles without the mercaptan and 0.8 moles with it. There was a corresponding change in rate of carbon dioxide formation, and carbon monoxide formation occurred at an appreciable rate, 0.25 moles.

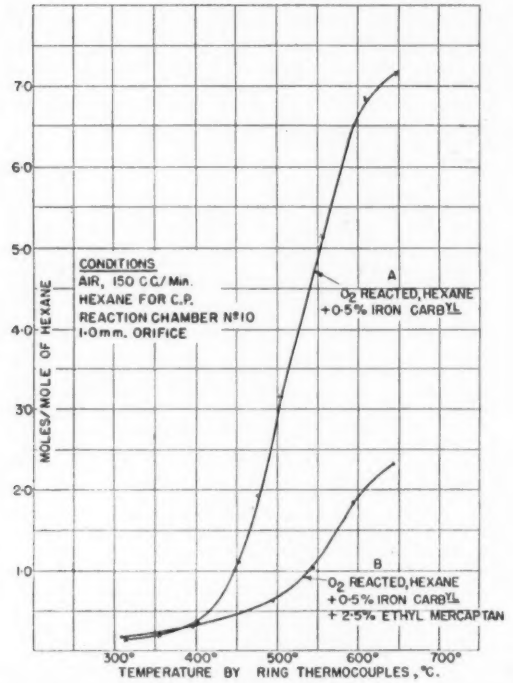

FIG. 9. *Graphs showing the inhibiting effect of ethyl mercaptan on the oxidation of hexane doped with iron carbonyl in glass reaction chamber (No. 10).*

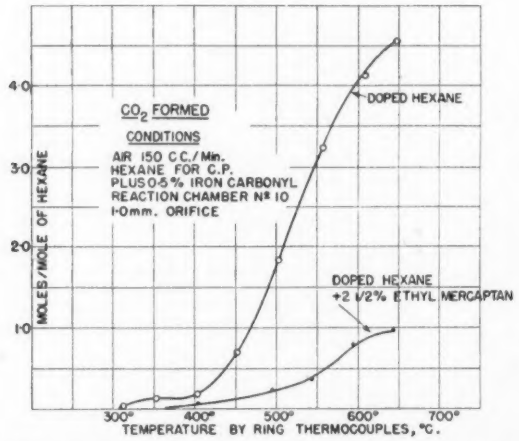

FIG. 10. *Graphs showing the effect of ethyl mercaptan on carbon dioxide formation when doped hexane is oxidized in glass reaction chamber (No. 10).*

The Effect of Ethyl Mercaptan on the Oxidation of Pentane in Reaction Chamber B, Fig. 11, Part II (9)

The reaction chamber was of mild steel which does not rust uniformly. The surface in contact with the reacting mixture of pentane–air would, therefore, comprise areas of iron oxide which would promote the formation of

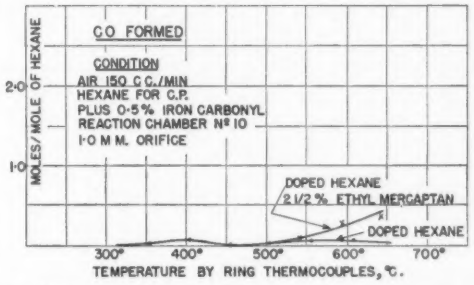

FIG. 11. *Graphs showing the effect of ethyl mercaptan on carbon monoxide formation when doped hexane is oxidized in glass reaction chamber (No. 10).*

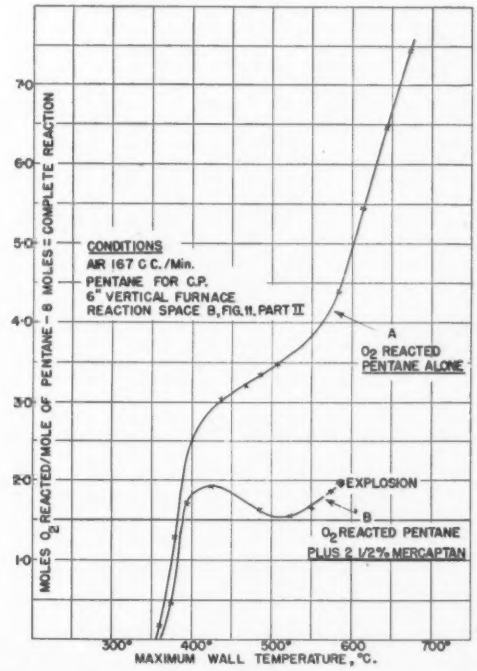

FIG. 12. *Graphs showing the effect of ethyl mercaptan on the oxidation of pentane (undoped) in a steel reaction chamber.*

partial oxidation products and areas of iron which would promote oxidation to final products, and, as there existed a steep temperature gradient over the length of the chamber, reaction can occur at a range of temperatures even when the maximum is 600° C. or over. The mixed nature of the reaction in the chamber is shown by Graph *A* of Fig. 12, which is characteristic of both the aldehyde and the carbon dioxide–steam reactions. The effect of the mercaptan is shown by Graph *B* of the figure. The carbon dioxide–steam reaction is nearly eliminated, as shown by the graphs of Figs. 13 and 14, leaving the aldehyde, which, in the absence of dilution with steam and carbon dioxide, ignites and explodes when the temperature reaches 590° C.

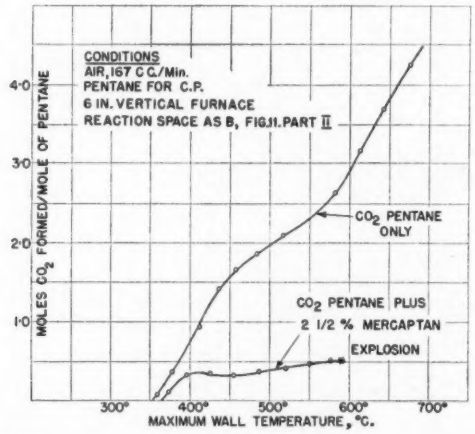

FIG. 13. *Graphs showing the effect of ethyl mercaptan on carbon dioxide formation when pentane is oxidized in the steel reaction chamber.*

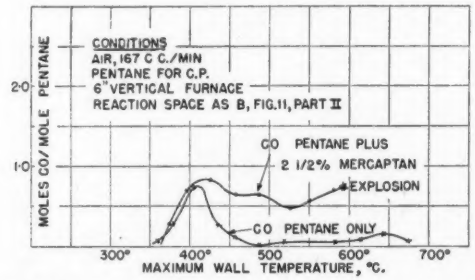

FIG. 14. *Graphs showing the effect of ethyl mercaptan on carbon monoxide formation when pentane is oxidized in the steel reaction chamber.*

Pentane Oxidation on Surface Remaining after the use of Mercaptan

An oxidation of pentane in the steel reaction space, as above, was carried out after the oxidation of pentane plus the mercaptan. The experimental

results are given by the graphs of Fig. 15 and show a considerable residual effect. Thus, for example, at 550° C., 3.8 moles of oxygen were reacted when the reaction chamber was first used. The rate dropped to 1.7 moles (Fig. 12) when the mercaptan was present and rose to 2.3 moles only on the residual surface (Fig. 15). The rates of carbon monoxide and carbon dioxide formation

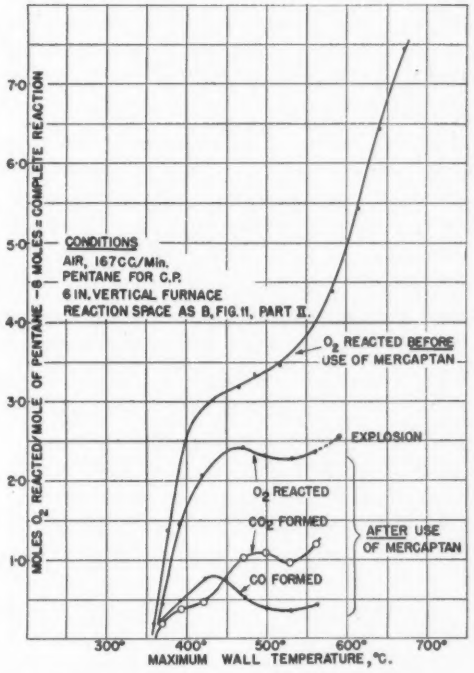

FIG. 15. *Graphs showing the effect of the surface of iron sulphide remaining* after *the use of ethyl mercaptan on the oxidation of pentane in the steel reaction chamber.*

on the residual surface are given by the lower graphs of Fig. 15 and comparing them with the graphs of Figs. 13 and 14 for pentane plus the mercaptan it will be seen that change of rates of carbon dioxide and carbon monoxide formation is in accordance with the changes in rates of oxygen reacted.

Conclusions

The experiments described in this section help to confirm that the oxidation of pentane is a heterogeneous reaction and show definitely that it is possible by the use of a catalyst poison to determine whether the antiknock action of metallic dope is due to the effect to inhibit the aldehyde reaction or the effect to promote reaction to final products.

Section V

The Pro-knock Effects of Lubricating Oils, Ethyl Mercaptan, and Hydrogen Sulphide

An extensive series of experiments, made some years ago by King and Moss (10, 11, 13, 14) demonstrated that lubricating oils when added to engine fuel varied in pro-knock effect. The greatest effect was obtained when the oils were added to doped fuels. Olein or oleic acid in a concentration of 1.0% only in the fuel destroyed almost the entire antiknock effect of nickel carbonyl in the large concentration of 10 cc. per gal. in the fuel, and was of similar effect in respect of iron carbonyl, but a concentration of 12% of olein in the fuel was required to destroy 85% of the antiknock effect of a 12 cc. per gal. addition to the fuel of tetraethyl lead. Castor oil, which is soluble in engine fuel to the extent of about 1½% only, was as effective as olein in destroying the antiknock value of nickel carbonyl, somewhat less effective in respect of iron carbonyl but was found to be of no *antiknock* effect when added to fuel doped with tetraethyl lead. The effect of the rape oils which are soluble in any concentration was similar to that of castor. The mineral oils were quite effective in reducing the antiknock value of the carbonyls but large additions were required in respect of tetraethyl lead even to obtain a relatively small effect.

The variation of the pro-knock effect with the natures of the oils and the metals of the dopes could not be explained in terms of theories based on homogeneous reactions, and little information was available about the effect of the several oils on heterogeneous reactions when the contact surfaces were of iron, nickel, or lead. It was known, however, that olein or oleic acid is a poison in respect of nickel catalysts (18, p. 909). Comment was made accordingly in the published account of the experiments and no further explanation attempted.

Similar engine experiments were made later using ethyl mercaptan and hydrogen sulphide additions to engine fuel instead of lubricating oils. The substances, added in small proportion, destroyed completely the antiknock effects of tetraethyl lead and iron carbonyl and gave rise to further pro-knock effects which will be described later. Again the experimental results could not be explained by accepted theory. The sulphur compounds were known catalyst poisons in respect of the carbon dioxide–steam reaction on iron and nickel surfaces, but an explanation of their pro-knock effect given accordingly would have involved the assumptions that catalytic surfaces were provided in the combustion space by the metallic dopes and that the steam formed on them in the absence of a catalytic poison was responsible for the antiknock effect. The experimental results were, therefore, withheld from publication until experiments could be made on the oxidation of hydrocarbon engine fuel at the high temperature of the 'end gas' and on the effect of sulphur catalyst poisons on the reactions.

Selection of Sulphur Catalyst Poison

It was a convenience to use a liquid compound soluble in engine fuel, and thiophene, carbon disulphide, and ethyl mercaptan were tried. The pro-knock effects of the compounds on straight run aviation petrol doped with 2.0 cc. per gal. of tetraethyl lead when used in the C.F.R. engine with unheated induction, are shown by the graphs of Fig. 16. It will be seen that all three

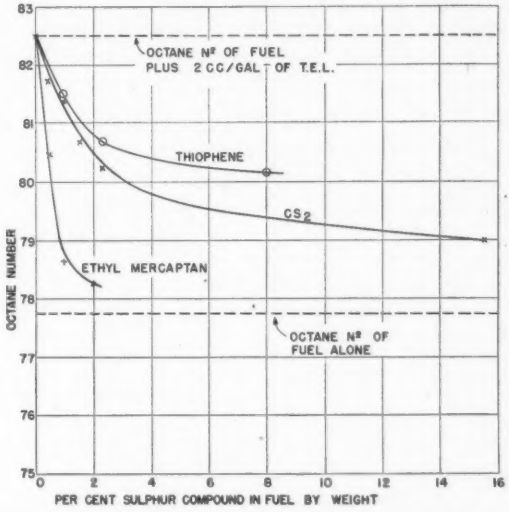

FIG. 16. *Graphs showing the pro-knock effect of three sulphur compounds when added to a low octane number engine fuel doped with tetraethyl lead. C.F.R. engine.*

substances were pro-knocks and that the mercaptan was outstanding, a concentration of 2% in the fuel being sufficient to destroy nearly all of the effect of tetraethyl lead to raise the octane number of the fuel from 77.8 to 82.5. It was not possible to prevent the loathsome odor of the mercaptan from reaching many parts of the building in which the engine was situated, and hydrogen sulphide was used as an equivalent catalyst poison for further experiments.

Engine Experimental Conditions

The experiments were made with a variety of hydrocarbon fuels used in the C.F.R. research engine. The intake air was unheated in some cases and in others raised to 300° F. as required for the standard method of determining octane number. The knocking tendency of the fuels was generally obtained by the H.U.C.R. method as used in the Air Ministry Laboratory (12), but sometimes by determining octane number. Some experiments were, however, made with very weak or very rich mixtures. Tetraethyl lead was used as the fuel dope in preference to iron carbonyl which on long continued use, especially in large concentration, destroys spark plug insulation and leaves

heavy deposits of oxide in the engine combustion space. The engine experimental results are given by a series of graphs and the experiments are described in succeeding paragraphs.

Hexane Experiments

Experiments with hexane as an engine fuel are of special interest because of the oxidation experiments described in Sections III and IV. The experiments were made with increasing additions of hydrogen sulphide when using either hexane alone or hexane plus 8.0 cc. of tetraethyl lead per gallon and when using correct, rich, and weak mixtures and heated and unheated air supply.

The experimental results are shown by a series of graphs, Fig. 17, H.U.C.R. being plotted against hydrogen sulphide additions. Mixture strengths are

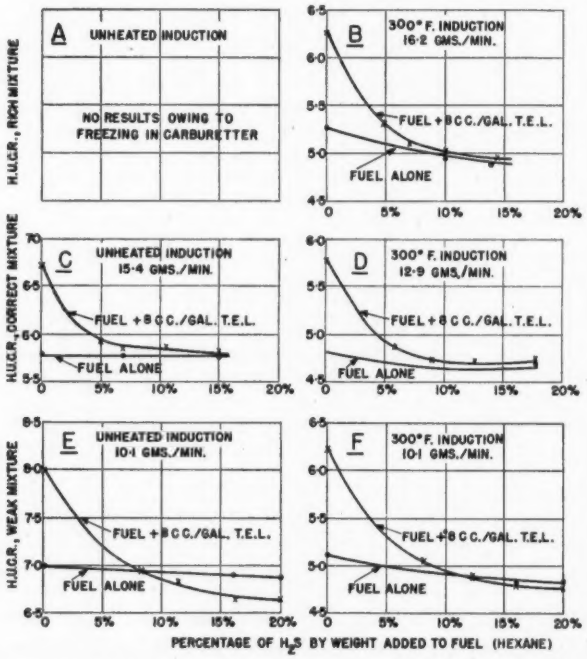

FIG. 17. *Graphs showing the pro-knock effect of hydrogen sulphide in respect of hexane doped with tetraethyl lead. C.F.R. engine, for correct, rich, and weak mixtures and heated (300° F.) and unheated induction.*

shown by the figures for fuel consumption in grams per minute. Considering first the results for correct mixture strength, Graphs *C* and *D*, it will be seen that with unheated induction, Graph *C*, H.U.C.R. increased from 5.75 to 6.7 on adding the dope to the pentane and then diminished to 5.9 when hydrogen sulphide was added in the concentration of 5%, that is, 85% of the antiknock

value of the dope addition was destroyed. On increasing the hydrogen sulphide addition even to the large concentration of 15% of the weight of the fuel, nothing more happened than the complete destruction of the antiknock effect of the metallic dope.

The engine would not run on hexane at high compression ratios without excessive detonation unless the mixture were very weak or very rich, and results of experiments made accordingly and with heated or unheated induction are given by the Graphs *B*, *E*, and *F* of Fig. 17. The graphs show that by using the hexane at a relatively high compression ratio or with a high induction temperature a small pro-knock effect in respect of the undoped fuel is obtained on adding hydrogen sulphide to the mixture. The explanation advanced is that in conditions of high inlet temperature and high flame temperature due to high compression ratio, the exhaust valve and/or other not well cooled surfaces, such as the piston crown, are raised to the temperature required for the partial oxidation of the end gas to steam and carbon dioxide. The temperature is about 400° C. according to the experiments with a steel reaction chamber, see Fig. 12, and on inhibition of the reaction by hydrogen sulphide the pro-knock effect shown by the experiments would be expected.

A further interesting effect is shown by Graphs *E* and *F*, fig. 17. The graphs cross, showing that the hydrogen sulphide after destroying the antiknock effect of the dope and that due to oxidation of the fuel on hot surfaces carries on to give rise to a further pro-knock effect. The 'further' effect increases as the compression ratio is raised and flame temperature increased accordingly. This will be discussed in succeeding paragraphs.

Aviation Fuel Experiments

The fuel was a high grade straight run Borneo petrol and when used undoped and with *unheated* induction the catalyst poisons were without the pro-knock effect which became evident when metallic dope was added. The experimental results given by the graphs of Fig. 18, obtained with an *induction temperature of 300° F.*, show the double effect already mentioned, that is, the hydrogen sulphide not only destroys the entire antiknock effect of the metallic dope but also the smaller similar effect attributed to the formation of steam and carbon dioxide on combustion chamber surfaces becoming active in the high temperature conditions. The two pro-knock effects obtained on adding hydrogen sulphide in a concentration of about 8% in the fuel suffice to reduce the octane number to 64.5.

Coal Spirit Experiments

The spirit was procured from the low temperature distillation of coal. The octane number was higher than that of the petrol but lead response was less; a 4 cc. per gal. addition of the metallic dope raised the octane number from 75½ to 84, whereas a similar addition to the aviation petrol raised the octane number from 70 to 85. The relatively low concentration of 3% of the hydrogen sulphide was sufficient to destroy the original antiknock effect of the dope, represented by an increase of 8½ octane numbers. The second and third

effects are as described for the engine experiments with hexane but are of greater magnitude and obtained with smaller percentages of hydrogen sulphide.

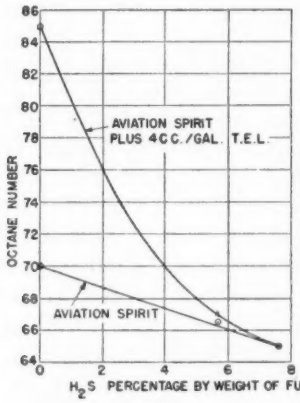

FIG. 18.

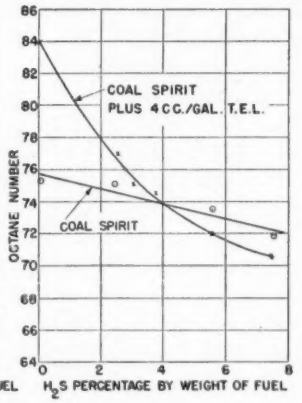

FIG. 19.

FIG. 18. *Graphs showing the pro-knock effect of hydrogen sulphide on aviation spirit doped with tetraethyl lead. C.F.R. engine, 300° F. induction temperature.*

FIG. 19. *Graphs showing the pro-knock effect of hydrogen sulphide on coal spirit doped with tetraethyl lead. C.F.R. engine, 300° F. induction temperature.*

Cyclohexane Experiments

The second and third pro-knock effects of hydrogen sulphide are shown by the engine experiments already described to become increasingly evident as the temperature of combustion is raised either by increasing compression ratio or by raising induction temperature. The experiments were, therefore, repeated in conditions requiring the use of compression ratios approaching the limit of the C.F.R. engine. Weak and rich mixtures of cyclohexane were used with induction temperatures of 300° F. and it will be seen by reference to the graphs of Fig. 20 that the highest useful compression ratios were 7.9 and 7.4 respectively in the circumstances. The H.U.C.R. values increased to 10.0 and 9.1 on adding tetraethyl lead to the fuel in the concentration of 24 cc. per gal. The excessive dope addition was used advisedly in an endeavor to determine whether the third pro-knock effect shown by the crossing of the graphs was due to the hydrogen sulphide or to the metallic dope in the presence of the sulphide. It is well known that the specific effect of tetraethyl lead diminishes as the concentration in engine fuel increases and it is probable that no more than one-third of the 24 cc. addition was effective to prevent detonation. The experimental results given by the graphs of Fig. 20 show that (*a*) The original increase in H.U.C.R. due to the dope was destroyed by about 5% of hydrogen sulphide in the weak mixture and about $2\frac{1}{2}$% in the rich mixture.

(*b*) The second pro-knock effect of the hydrogen sulphide is more pronounced than in the experiments made at lower compression ratios. Thus the sulphide

in the concentration of 15% reduced the H.U.C.R. of the undoped fuel, weak mixture, from 7.9 to 6.2, that is, an amount equal to 80% of the increase obtained with the large addition of dope to the fuel in the absence of the catalyst poison. Similar effects were obtained for the rich mixture but comparisons cannot be made because the percentage of hydrogen sulphide added to the fuel–air mixture was not large enough to obtain limiting values for the effects.

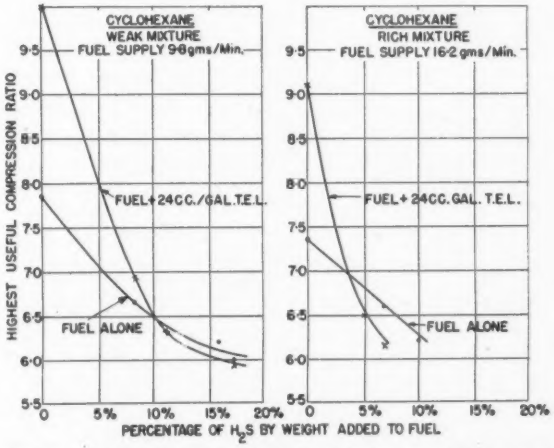

FIG. 20. *Graphs showing the pro-knock effect of hydrogen sulphide on rich and weak mixtures. Cyclohexane doped with tetraethyl lead. C.F.R. engine, 300° F. induction temperature.*

(*c*) The third pro-knock effect of the hydrogen sulphide, shown by the crossing of the graphs, is most apparent for the rich mixture for which fuel was supplied at the rate of 16.2 gm. per min. as compared with 9.8 for the weak mixture. The rich mixture in the engine contained, therefore, nearly twice as much dope as the weak mixture, and, since conditions in respect of hydrogen sulphide supply remained unchanged, it is reasonable to suppose that the third pro-knock effect was due in some way to the tetraethyl lead when used at high flame temperatures in the presence of the sulphide.

Benzene Experiments

The experiments with benzene as the engine fuel were undertaken to confirm that, as suggested above, tetraethyl lead becomes a pro-knock if used in conditions of high flame temperature and in the presence of hydrogen sulphide. It is well known that benzene does not detonate in the C.F.R. engine even when the compression ratio is as high as 10. The addition of metallic dope is, therefore, without apparent effect. Experiments were made accordingly at an induction temperature of 300° F. The experimental results are given by the graphs of Fig. 21. They show that when the undoped fuel was used at the high compression ratio of 10, detonation did not occur until hydrogen sulphide had been added to the mixture in the high concentration of nearly 20% by

weight of the benzene. This result confirms that the 'further' pro-knock effect observed in the earlier experiments was not due to the effect of the hydrogen sulphide alone. It will be noted, however, that on adding tetraethyl lead to the benzene in the concentration of 4 cc. per gal. detonation occurred

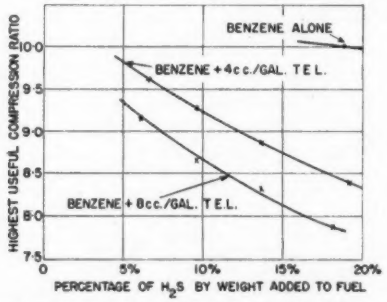

FIG. 21. *Graphs showing the pro-knock effect of tetraethyl lead in small concentration in benzene obtained when combustion occurs in the presence of hydrogen sulphide. C.F.R. engine, unheated induction.*

and the H.U.C.R. fell to 9.6 when the percentage of hydrogen sulphide in the fuel was 7% only, and further to 8.4 when the percentage was increased to 19. On increasing the concentration of tetraethyl lead to 8 cc. per gallon, a still greater pro-knock effect was obtained as shown by the lower graph of the figure, H.U.C.R. being reduced to 9.1 when the percentage of hydrogen sulphide was about 7, and to 7.8 when it was 18.

Section VI

Discussion of Experimental Results

Nature of the Reactions Described

It was suggested, Section V, Part II (9), for discussion and further experiment "that the oxidation of pentane is an heterogeneous reaction, velocity depending on the nature of the contact surface and the manner in which the reacting mixture is brought into contact with it". Some further experimental work supporting the general proposition has been described in this Part and will be discussed in succeeding paragraphs.

When pentane is oxidized in reaction chamber No. 10, and the active surface is of clean Jena glass, the usual aldehyde hump appears on the graph for oxygen reacted, see Fig. 3. If now iron carbonyl be added to the reacting mixture, giving an active surface of iron, the aldehyde hump disappears and reaction is almost completely to steam and carbon dioxide (Graph *B* of the figure). Then on allowing the active surface to go to iron oxide by ceasing to supply the carbonyl, the aldehyde hump reappears (Graph *C* of the figure). These experimental results obtained with a flow configuration especially adapted rapidly

to remove oxidation products from the surface on which they are formed give strong support to the view that the oxidation reaction is heterogeneous.

The experiments with ethyl mercaptan give further support to the view mentioned. Thus, the graphs of Fig. 6 for the oxidation of hexane in the glass reaction chamber No. 10 in clean condition show that the aldehyde hump remains when ethyl mercaptan is added to the reacting mixture, indicating that the mercaptan is without effect to alter the surface at the temperature of the experiments. If, however, iron carbonyl be added to the reacting mixture instead of the mercaptan, Fig. 9, and an active surface of iron is deposited on the glass, the aldehyde hump disappears, and oxidation is to carbon dioxide and steam at all temperatures of reaction, as for pentane in similar conditions. If now the active iron surface is converted to the sulphide on adding the mercaptan, the aldehyde hump is not recovered and reaction velocity at all temperatures diminishes to a relatively low rate. The rate may reach zero at temperatures rising to over 500° C. and be inappreciable at higher temperatures in *suitable conditions of flow configuration* as shown by Graph *B*, Fig. 18, Part II (8). Again it is difficult to interpret the experimental results otherwise than by considering the reactions to be heterogeneous.

The experiments with the steel reaction chamber of relatively large volume, Fig. 12, are also significant. The surface was not of uniform temperature and was in part of iron oxide and in part of iron. The oxidation of pentane in the chamber is, therefore, a mixed reaction yielding, over the low temperature range, aldehyde on the oxide and final products on the iron. On adding ethyl mercaptan to the mixture, the reaction to aldehyde which occurs on the surface of iron oxide as previously shown, Fig. 3, continues but that to steam and carbon dioxide occurring on the iron part of the surface is inhibited, giving the over-all result as shown by the graphs of Fig. 12.

The Negative Temperature Coefficient of Reaction

The negative coefficient depends for its existence on the aldehyde hump which is more pronounced for oxidations in reaction chamber No. 10 than for oxidations in conventional combustion reaction spaces. It is necessary, therefore, in seeking an explanation to remember that in reaction Chamber No. 10 oxidation occurs with great rapidity, time of exposure being of the order of one second, that the high velocity of reaction is due to the flow configuration being adapted to remove surface oxidation products immediately on their formation, that the products do not remain in the chamber and on leaving it to reach the exit are exposed to temperatures lower than that of their formation. The chamber is thus suitable for following the course of reactions occurring at specified temperatures, and the existence of a negative temperature coefficient when pentane is oxidized in the chamber on other than a metal surface indicates that a reaction of high velocity is succeeded, on raising the temperature, by one of lower velocity. Thus referring to the graphs of Fig. 10, Part I (8), it will be seen that carbon monoxide formation reaches a maximum at about 400° C., the temperature of the aldehyde hump,

and then falls to a minimum at 520° C. The rate of carbon monoxide formation then rises as temperature increases, slowly at first but with great rapidity at temperatures above 600° C. until at 660° C. it exceeds that of carbon dioxide formation. The experimental results show a high reaction velocity over the lower temperature range when the pentane is oxidized to aldehyde, and initially a relatively low reaction velocity at higher temperatures when the pentane is oxidized to carbon monoxide. During the temperature interval between the high velocity, low temperature reaction to aldehyde and the acceleration to high velocity of the high temperature reaction to carbon monoxide, the over-all rate of oxygen reacted reaches the relatively low value which accounts for the negative temperature coefficient. The oxidation of pentane could not proceed in the manner described if the reactions were homogeneous, so conversely the existence of a negative temperature coefficient may be taken as an indication of heterogeneous reactions.

A negative temperature coefficient of reaction was obtained by Pease (16) nearly 20 years ago and was observed when propane was oxidized by a flow method. The experiments of Pease and those of later workers are discussed by Jost in *Explosion and Combustion Processes in Gases*, pages 427–449, English translation by Huber O. Croft, and it is stated, page 449, that many facts support the conclusion that the reaction mechanism differs as between low and high temperature.

Oxidation in the End Gas in an Engine

If oxidation of the fuel in the end gas be a heterogeneous reaction, the antiknock effect of metallic dope can be attributed to catalytic action, and detonation in the body of the gas cannot be due primarily to any process of oxidation. If, on the other hand, oxidation be a homogeneous reaction, existing theory in respect of the cause of detonation and the antiknock action of metallic dope would be supported. Antiknock action only will be discussed at present although some of the experimental work described in Parts I (8) and II (9) has an important bearing on the cause of ignition and detonation; a subject which it is proposed to discuss in a subsequent Part.

The Revised Positive Catalyst Theory

It is well established that the unburned combustible mixture (end gas) confined between the advancing flame and some part of the surface of the engine combustion space is the seat of detonation, that the *metal* of organometallic compounds is responsible for their antidetonation effect, and that turbulence is effective to reduce detonation. The temperature of the end gas in an engine not supercharged and using unheated mixture has been calculated to increase from 670° C. for a compression ratio of 4 to 880° C. for a compression ratio of 10, while the flame temperature may rise accordingly from 2500° to 2750° C., and radiation would be expected to give rise momentarily to high surface temperatures. The conditions as described, although prevailing for a small fraction of a second, suffice for at least partial oxidation of the end gas by flameless combustion. The experimental results for the

laboratory oxidation of doped pentane or hexane given by the graphs of Fig. 8, Part I (8), and Fig. 5 of this Part, in conditions of temperature, turbulence, and time of reaction approaching those prevailing in the combustion space of an engine, show that the metal of the dope deposited on the available surface effects the oxidation of the hydrocarbons to steam and carbon dioxide with great rapidity at temperatures above 500° C., as long as the metal surface is maintained by continuous deposition from the organometallic compound. The end gas in an engine is always at a temperature higher than 500° C. late in the period of flame travel and the contact surface of the gas probably at a still higher temperature due to radiation from the flame. Doped fuel in the end gas would, in the circumstances, be oxidized with great rapidity to steam and carbon dioxide on the catalytic surface maintained as in the laboratory reaction chamber by continuous deposition of the metal of the dope. The effect of steam especially to reduce inflammability is shown by the experiments described in Section I, and, dilution of the end gas being sufficient, self-ignition ahead of the flame could not occur and combustion would be completed in the normal course without detonation. Verification that the action of metallic dope is as described is obtained by considering the effects of sulphur compounds which are catalytic poisons in respect of the carbon dioxide – steam reaction on metal surfaces. Thus, ethyl mercaptan inhibits the carbon dioxide–steam reaction in laboratory oxidations of doped fuels, as shown by the experiments described in Section IV, and the mercaptan or hydrogen sulphide, when added to doped engine fuel, destroys the antiknock effect of the organometallic compounds as shown by the experiments described in Section V. When hexane undoped was used as fuel in the C.F.R. engine in conditions leading to relatively low flame and engine temperatures, the catalytic poison was of no pro-knock effect even in the large concentration of 15% in the fuel, Graph *C*, Fig. 17. However, when conditions of engine operation led to relatively high surface temperatures in the combustion space, the consequent steam–carbon dioxide reaction became an antiknock factor because knock occurs on its inhibition by the catalyst poison (see Figs. 18, 19 and 20). The antiknock effect of hot surfaces is most evident at high compression ratios which are necessarily accompanied by correspondingly high flame temperatures. Thus, referring to Fig. 20, inspection of the graphs shows the decrease in H.U.C.R. obtainable by inhibition of the carbon dioxide–steam reaction to be almost as great as the increase obtained on the addition of tetraethyl lead in very large concentration. That is, the tetraethyl lead antiknock effect is added to an existing effect. Both effects are due to dilution of the end gas with the product of a carbon dioxide–steam reaction and both are destroyed by the catalyst poison.

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